



Ultra-Rapid Analysis of High Molecular Weight Compounds Using SEC Mode

Introduction

Size Exclusion Chromatography (SEC) is one of the High Performance Liquid Chromatography (HPLC) separation modes where analytes are separated based on their sizes. The technique is frequently used to analyze high molecular weight compounds, as SEC mode is effective evaluating their physical properties. However, the longer analysis time of SEC is a drawback. Consequently, the eluent consumption of a SEC analysis is increased. Since organic eluents used for Gel Permeation Chromatography (GPC) are expensive and cause environmental concerns, the use of organic solvents is a GPC related problem. Therefore, we have worked on the development of novel, ultra-rapid analysis GPC columns which have following characteristics: suppressed pressure elevation under fast flow rate, high durability, wide working range suitable for molecular weight distribution analysis, and high tolerance to the repeated eluent replacement. This newly developed Shodex GPC HK series columns successfully reduced the analysis time and the eluent consumption. This technical article introduces the characteristics and the applications of Shodex GPC HK series columns.

Specifications of Shodex GPC HK series columns

The Shodex GPC HK series offers three columns with different working ranges. Table 1 summarizes their specifications. All has column size of 4.6 mm I.D. x 150 mm L. A corresponding guard column is available. In addition to the regular HK series columns, HK-HFIP404L is specifically designed for the analysis using HFIP eluent. Figure 1 shows the calibration curves of HK-401, HK-404L, and HK-405 obtained using THF eluent.

Table 1 Specifications of Shodex GPC HK series columns

HK series columns

Product name	Exclusion limit (MW)	Plate number (TP/column)	Particle size (μm)	Shipping Solvent
GPC HK-401	2,000*1	9,000	3	THF
GPC HK-404L	1,000,000*1	9,000	3.5	THF
GPC HK-405	4,000,000*1	7,000	3	THF
GPC HK-HFIP404L	200,000*2	9,000	3.5	HFIP

1 Polystyrene *2 PMMA

Guard filter

Product name	Contents
GPC HK-G	One holder and one filter
GPC HK-G filter	3 filters

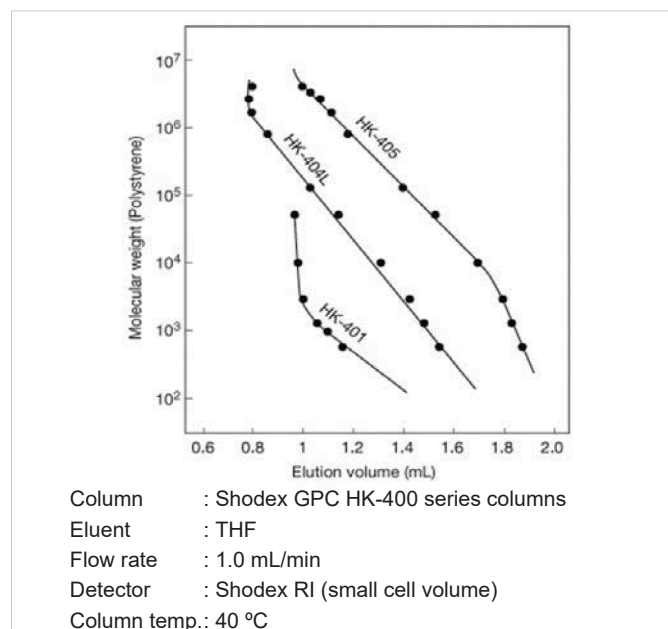


Fig. 1. Calibration curves of the Shodex GPC HK series columns

Characteristics of Shodex GPC HK Series Columns

1. The novel styrene divinylbenzene copolymers of a uniform monodisperse particles

The size of packing material filled in the HK series columns are uniformly controlled at 3 or 3.5 μm (Fig. 2). They provide mechanical strength and high tolerance to maximum of 2.0 mL/min flow rate. This contributed to the significantly shorten analysis time of the HK series columns compared to that of previously available standard GPC columns.

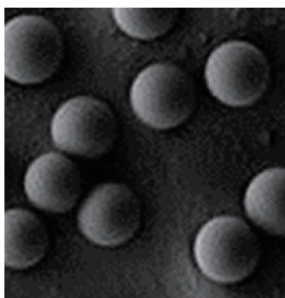


Fig. 2 Scanning electron microscope image of HK-404L packing material

Figure 3 shows example chromatograms of the rapid analysis. The chromatograms were obtained from the analysis of polycarbonate and polystyrene molecular standards under the maximum-working flow rate, 2.0 mL/min. The column pressure observed was about 26 MPa at 40 °C and their analysis times were both within 2 minutes. Even with this ultra-rapid analysis condition, polystyrene standards with different molecular weights were well separated.

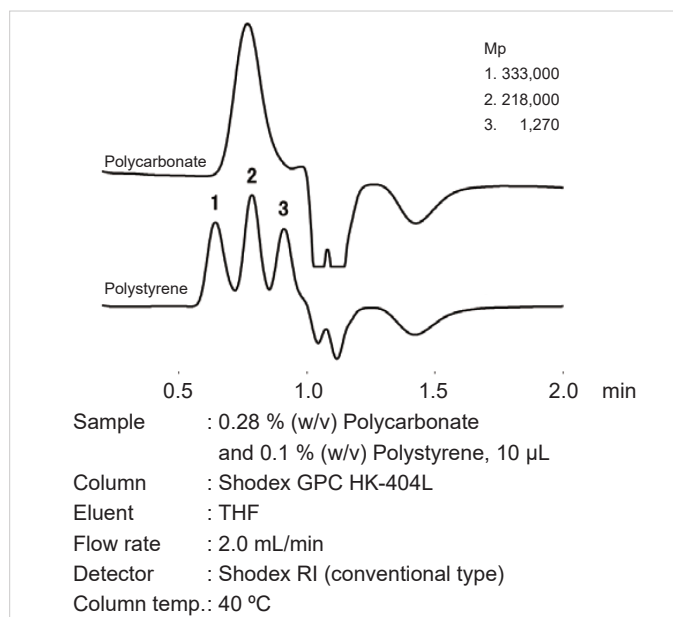


Fig. 3 Chromatograms of polycarbonate and polystyrene

The HK series columns can be used the same as full sized GPC columns for the molecular weight distribution determination. Figure 4 shows comparison of a regular GPC column, KF-805L, and the new HK-404L column for the molecular weight distribution analysis of poly(isobutyl methacrylate). Polystyrene was used as molecular weight calibration standard. The result demonstrated that the use of HK-404L reduced the analysis time and the eluent consumption down to approximately one-sixth of the regular GPC column, while both obtained similar molecular weight distribution analysis results. This proved that even under ultra-rapid analysis conditions, the HK series column can obtain reliable calibration curves using a set of molecular weight standards, and effectively determine the molecular weight distribution of the analytes, in the same manner as regular GPC columns.

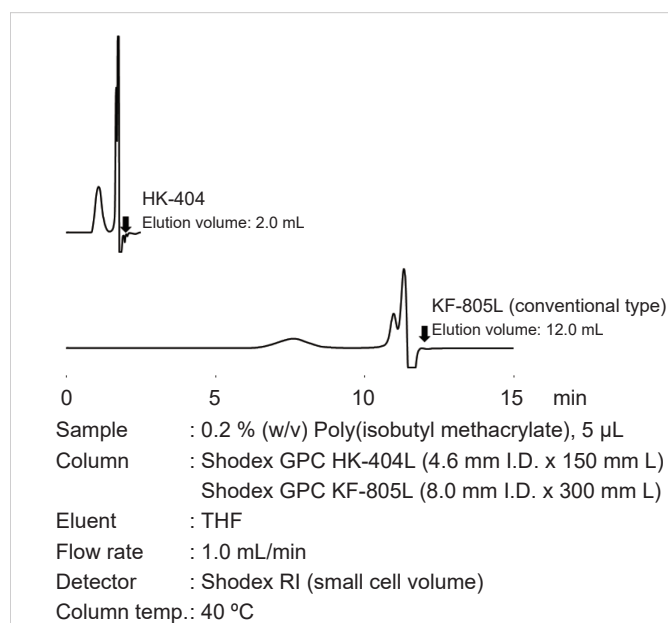


Fig. 4. Comparison of HK-404L and KF-805L for the molecular weight distribution analysis of poly(isobutyl methacrylate)

2. No need to designate a column for an application

GPC HK series columns can be used with THF, toluene, chloroform, and DMF. The columns have high tolerance to repeated replacement among the listed eluents. Therefore, unlike other GPC columns, it does not require to designate a column for a certain eluent.

3. Ultra-rapid analysis using a standard HPLC system

The HK series columns may be considered as semi-micro size GPC columns because of its 4.6 mm internal diameter and 150 mm column length. However, the column can be used with both standard HPLC system and semi-micro HPLC system with reduced dead-volume. As long as the system consists of a required detector and tolerance towards the eluent to be used, the column does not limit the HPLC system to be used with. Both systems achieve similar molecular weight distribution results. The Ultra-High Performance Liquid Chromatography (UHPLC), currently available in the market, is designed to work under ultra-high pressure conditions. However, as mentioned earlier, the HK series columns are filled with monodisperse particles. This keeps the system and the column pressures low even when used at high flow rate, and thus they do not require to be used with UHPLC system.

Figure 5 shows comparisons of standard and semi-micro HPLC systems for the molecular weight distribution analysis of styrene butyl methacrylate copolymer and polystyrene. The chromatograms and the obtained average molecular weight distribution results demonstrated that the column provided similar results for both systems.

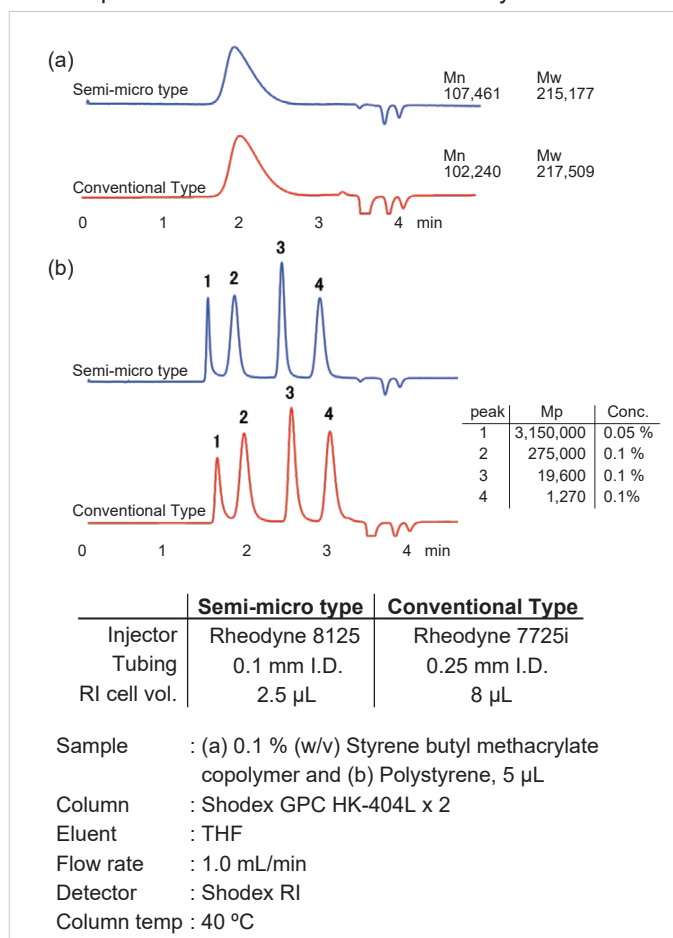


Fig. 5. Comparison of standard and semi-micro HPLC systems for the molecular weight distribution analysis of (a) styrene butyl methacrylate copolymer and (b) polystyrene standards

Application Examples

1. Column combination and the improved separation

A high-molecular weight polymer with a wide range distribution can be analyzed by HK-404L or HK-405 (depends on its molecular size and range). However, sometimes components of the eluent overlap with the analyte peak and influence the measurement. In such cases, combining HK-401, which has an exclusion limit of 1,000 and offers good separation in the lower molecular weight range, can improve the separation of the eluent originated peaks from the analyte peak.

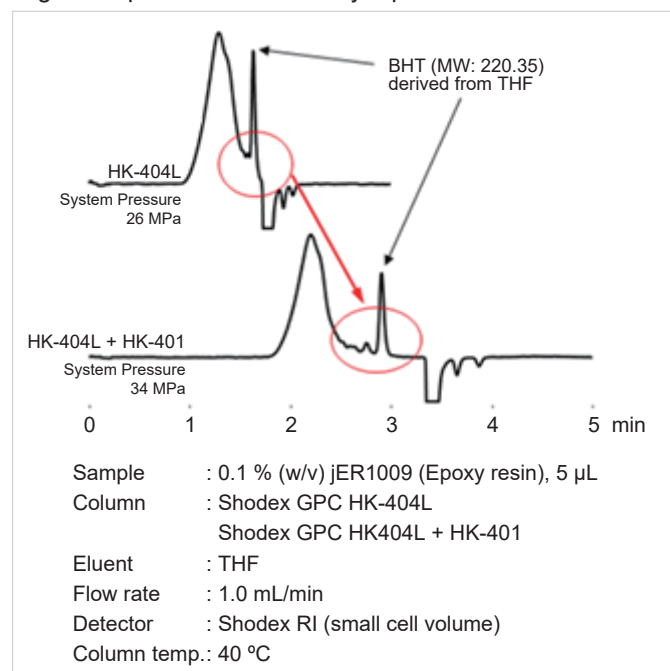


Fig. 6. Separation of eluent peak from the analyte peak

2. Analysis using DMF eluent

Figure 7 shows an example analysis using DMF eluent. The analyte in this example was poly(4-vinylpyridine), a basic high molecular weight compound. The ionic interaction may occur when DMF alone is used as an eluent. This causes adsorption of analyte to the stationary phase. The ionic interaction can be suppressed by adding lithium bromide to the DMF eluent. The obtained chromatogram proved the smooth elution and the good separation of the analyte.

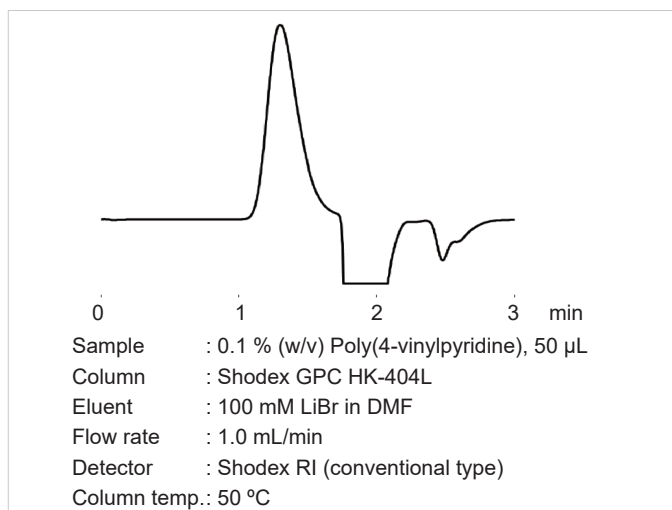


Fig. 7. Chromatogram of poly(4-vinylpyridine)

3. Absolute molecular weight determination of a star-shaped polystyrene by MALS

The HK-400 series column is also applicable working with multi-angle light scattering detector (MALS) for the absolute molecular weight determination. Figure 8 shows the absolute molecular weight determination of a star-shaped polystyrene using HK-404. The obtained absolute molecular weights were $M_n = 348,200$ and $M_w = 390,100$. The rms radius was 15 nm. These results confirmed the column's ability to provide a rapid and accurate absolute molecular weight.

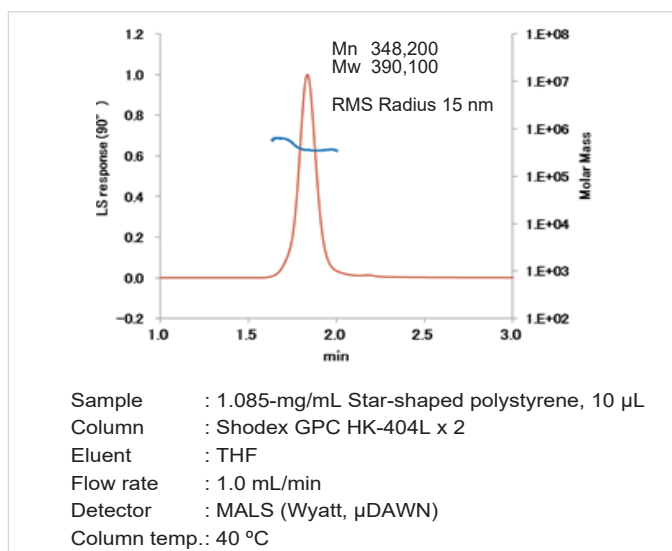


Fig. 8. Absolute molecular weight determination of a star-shaped polystyrene

4. Analysis of nylon using HFIP

HK-HFIP404L is specifically designed to be used with HFIP eluent. Figure 9 shows comparison chromatograms of HK-HFIP404L and the conventional semi-micro GPC column, LF-404, for the analysis of Nylon 11. By using the HK-HFIP404L, the analysis time was reduced to half of the conventional semi-micro GPC column.

The eluent consumption was also reduced to 2.25 mL per analysis. Both provided similar molecular weight distribution results. HFIP is an expensive solvent, therefore the HK-HFIP404L column demonstrated its advantages in analysis time and eluent consumption. Thus, the use of HK-HFIP-404L can be said as an environmental friendly and cost-saving method.

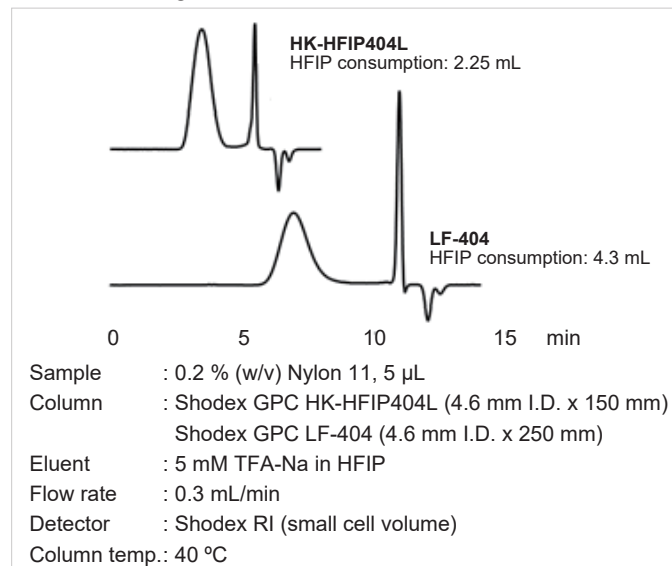


Fig. 9. Comparison chromatograms of Nylon 11 analyzed by HK-HFIP404L and LF-404

Conclusions

The Shodex GPC HK series columns are mechanically stronger than the previously available GPC columns even under ultra-rapid analysis conditions. The HK series columns provide reliable and similar molecular weight distribution determination results obtained by regular GPC columns. The columns are not required to be used with a special HPLC system, such as UHPLC, but can be used with both standard and semi-micro HPLC systems. The applications introduced in this technical article demonstrated the effectiveness of the HK-400 series columns for the ultra-rapid GPC analysis which contribute to increased analysis speed and reduced organic solvent consumption.

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Figures and descriptions in this article are provided to help you select appropriate columns. However they do not guarantee nor warrant the suitability for your applications.

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