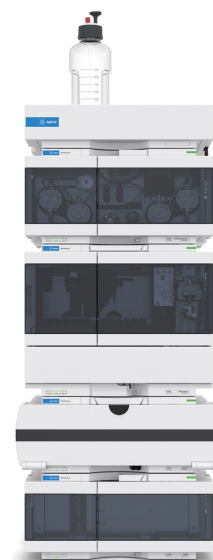
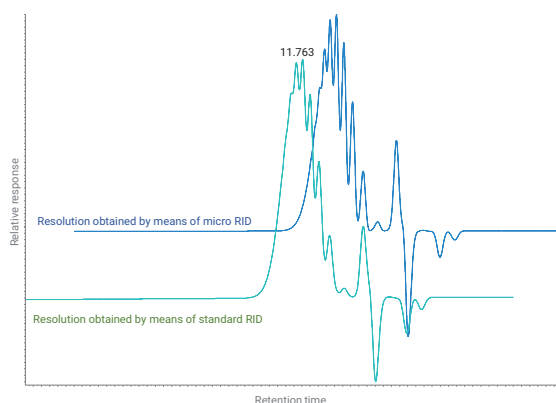


Improved GPC with the Agilent 1290 Infinity II Micro RID

Suitable for Agilent
1290 Infinity III LC

Highest throughput at lowest solvent consumption
with best-in-class resolution



Author

Edgar Naegele
Agilent Technologies, Inc.

Abstract

This Application Note describes the advantages of using the Agilent 1290 Infinity II LC with the Agilent 1290 Infinity II Micro Refractive Index Detector (RID) for GPC. The Micro RID enables the use of GPC columns with narrow inner diameters, such as 4.6 and 2.1 mm. While the system still allows the operation of the typically used 7.5 mm id columns, the use of narrow id columns will lead to better resolution, faster run time, and lower solvent consumption at the same or even better polymer separation performance.

Introduction

Typical GPC standard methods are based on long columns with larger inner diameters (id)(for example, 75 mm), with separations conducted at higher flow rates, such as 1 mL/min and above. As a result, there is a high consumption of expensive solvents such as THF. To overcome this drawback, modern GPC methods use columns with lower ids, 4.6 mm or even 2.1 mm, of the same length, which can be operated at reduced flow rates. To achieve enough precision for the flow rate, modern UHPLC pumps are required. The ability of these pumps to work at very high pressure could be useful for column phases that are based on silica material, enabling coupling in multiple series. Modern GPC stationary phase materials also accommodate elevated flow rates. To maintain resolution, detectors with very low cell dead volumes are required.

This Application Note demonstrates the advantage of using the 1290 Infinity II LC for GPC. The featured Agilent 1290 Infinity II High-Speed Pump can deliver very low flow rates with the highest precision necessary for the accurate determination of molecular weights. The Micro RID enables the use of GPC columns with narrow ids down to 2.1 mm, while maintaining highest resolution. This combination gives better resolution, faster run times, and lower solvent consumption at the same or even better separation performance compared to classical GPC methods, which can be operated using the same system configuration.

Experimental

Agilent 1290 Infinity II GPC/SEC system

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Vialsampler (G7129B)
- Agilent 1260 Infinity II Multicolumn Thermostat (MCT) (G7116A)
- Agilent 1290 Infinity II Micro RID (G7162B) or
- Agilent 1260 Infinity II RID (G7162A)

Software

- Agilent OpenLab v2.3 and the GPC add-on (G7860AA)

Method

Parameter	Value
Solvent	THF, isocratic, channel B
Flow Rates	0.06, 0.3, 0.6, 1.0 mL/min
Stop Time	22, 11, or 7 minutes
Column Temperature	35 °C, 2 columns in series connected with 75 µm capillary (105 mm length)
Injection Volume	20 µL, 4 µL
RID	Optical unit temperature: 35 °C Data rate: 18 Hz Signal polarity: positive

Columns

- Agilent PLgel MiniMixE, 250 × 4.6 mm, 3 µm (PL1510-5300)
- Agilent PLgel MixedE, 300 × 7.5 mm, 3 µm (PL1110-6300)
- Agilent InfinityLab OligoPore, 250 × 2.1 mm (PL1913-5520)

Calibration

Agilent EasiVial, PS-L 2 mL (PL2010-0401):

- **Red vial** (Mp: 47,190, 9,960, 2,980, 580 g/mol)
- **Green vial** (Mp: 30,230, 7,640, 1,840, 370 g/mol)
- **Yellow vial** (Mp: 18,340, 4,900, 935, 162 g/mol)

Samples

- Polystyrene 580
- Polystyrene 1320
- Epoxy resin (Epikote)

Chemicals and solvents

- THF, tetrahydrofuran ≥99.9% inhibitor-free, CHROMASOLV Plus for HPLC, Honeywell Riedel-de Haën (Seelze, Germany)

Results and discussion

As an initial experiment, a set of polystyrene calibration samples was measured on typical standard GPC/SEC columns (7.5 × 300 mm) at a flow rate of 1 mL/min with the Micro RID and the standard RID. The resulting separation obtained on the Micro RID of one calibration mixture (red capped vial with low molecular weight calibration compounds, see Experimental) is shown in Figure 1. The chromatogram shows a clear separation of all components where the low molecular weight component is partially resolved, and shows the comprising polystyrene oligomers. The same result was obtained with the standard RID (data not shown). The difference between the Micro RID and the standard RID is their cell volume of 2 and 8 μ L, respectively. At typical standard GPC/SEC conditions, large id columns, and high flow rates, this difference has no influence on the resolution. To prove that the results for both detectors are identical, the molecular weight (Mp, Mn, Mw, and PD) of a lower molecular polystyrene (Mp 1,320) was determined (Figure 2). It shows that, for both detectors, the resolution was identical, and the molecular weight information was comparable.

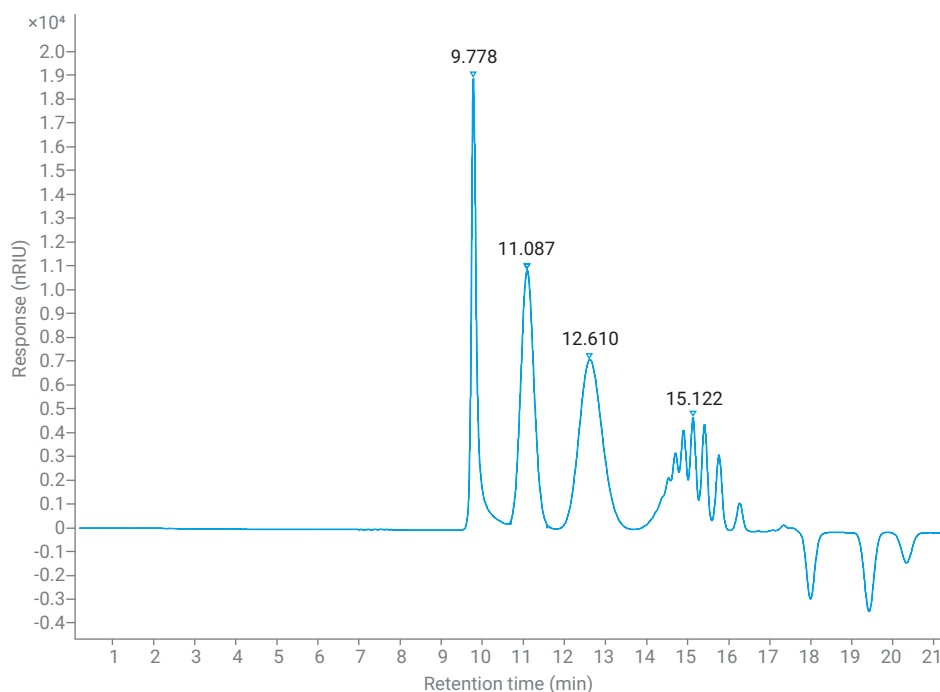


Figure 1. Separation of a low molecular weight polystyrene mixture (red capped vial with low molecular weight calibration compounds, see Experimental) on an Agilent PLgel column (two 300 × 7.5 mm, 3 μ m) at a flow rate of 1 mL/min THF with detection on an Agilent 1290 Infinity II Micro RID.

Results Set	RT (min)	Mp (g/mol)	Mn (g/mol)	Mw (g/mol)	PD
GPC-Standard-RID	13.805	1,314	1,048	1,265	1.21
GPC-micro-RID	13.740	1,345	1,065	1,254	1.18

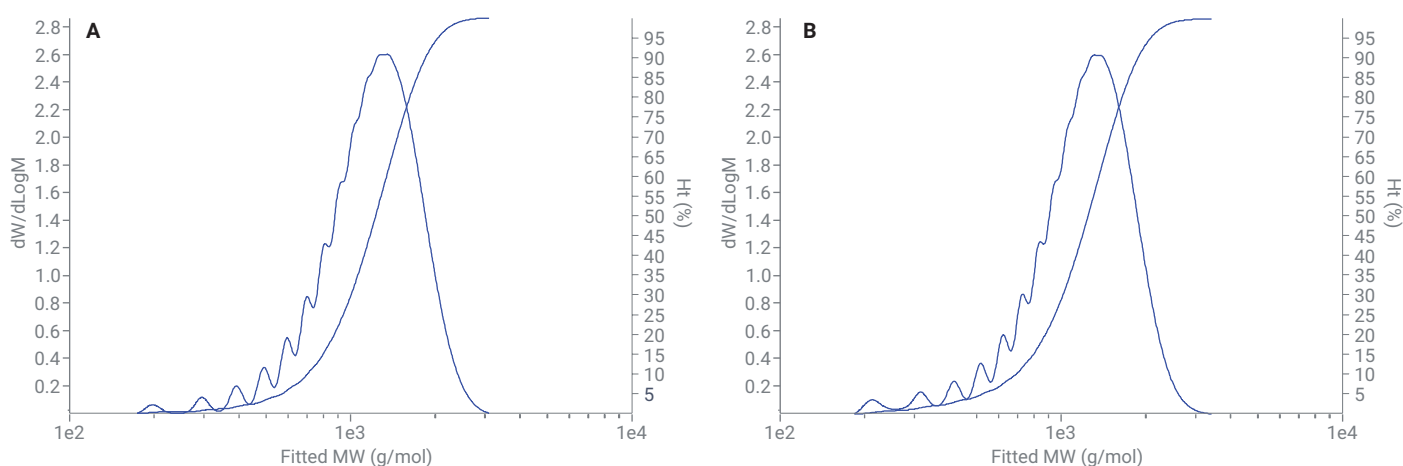


Figure 2. Determination of molecular weight of a low molecular weight polystyrene with the Agilent 1290 Infinity II Micro RID (A) and the Agilent 1260 Infinity II standard RID (B) on an Agilent PLgel column (two 300 × 7.5 mm, 3 μ m) at a flow rate of 1 mL/min THF (Mn: number average molecular weight, Mw: weight average molecular weight, Mp: molecular weight at peak maximum, PD: polydispersity index).

The drawback of using the typical GPC/SEC standard conditions is the associated large solvent consumption of 1 mL/min or more. This flow rate produces a large amount of harmful solvent waste and high solvent and waste disposal costs. To overcome this drawback, a set of experiments at different flow rates was performed with columns of 4.6 mm id to demonstrate the comparability of the results. The initial flow rate was calculated downwards from the standard conditions to obtain retention times comparable to the calibration standards. Then, the flow rate was accelerated to increase the throughput.

Figure 3 shows the comparison of the results achieved with different flow rates at lower solvent consumption or higher throughput using the Micro RID. For instance, at a flow rate of 0.3 mL/min on the 4.6 mm id column, the same run time was achieved as observed under the standard conditions listed. The resolution of the lower molecular weight compounds in the calibration mixture (red capped vial with low molecular weight calibration compounds, see Experimental) is of the same quality (Figures 3A1 and 3A2). To increase the throughput, the flow rate was increased to 0.6 mL/min (Figures 3B1 and 3B2) and 1.0 mL/min (Figures 3C1 and 3C2).

The increase to 0.6 mL/min exactly doubled the possible throughput, with identical resolution for the low molecular weight components. A further increase in flow rate to 1 mL/min increased the throughput approximately three times compared to the initial conditions. The resolution of the low molecular weight components was still good, but slightly compromised in terms of higher molecular weight compounds (lower retention time, Figures 3C1 and 3C2). The solvent consumption per sample was reduced to approximately 30% for all cases (Table 1) compared to the standard conditions on a 7.5 mm id column.

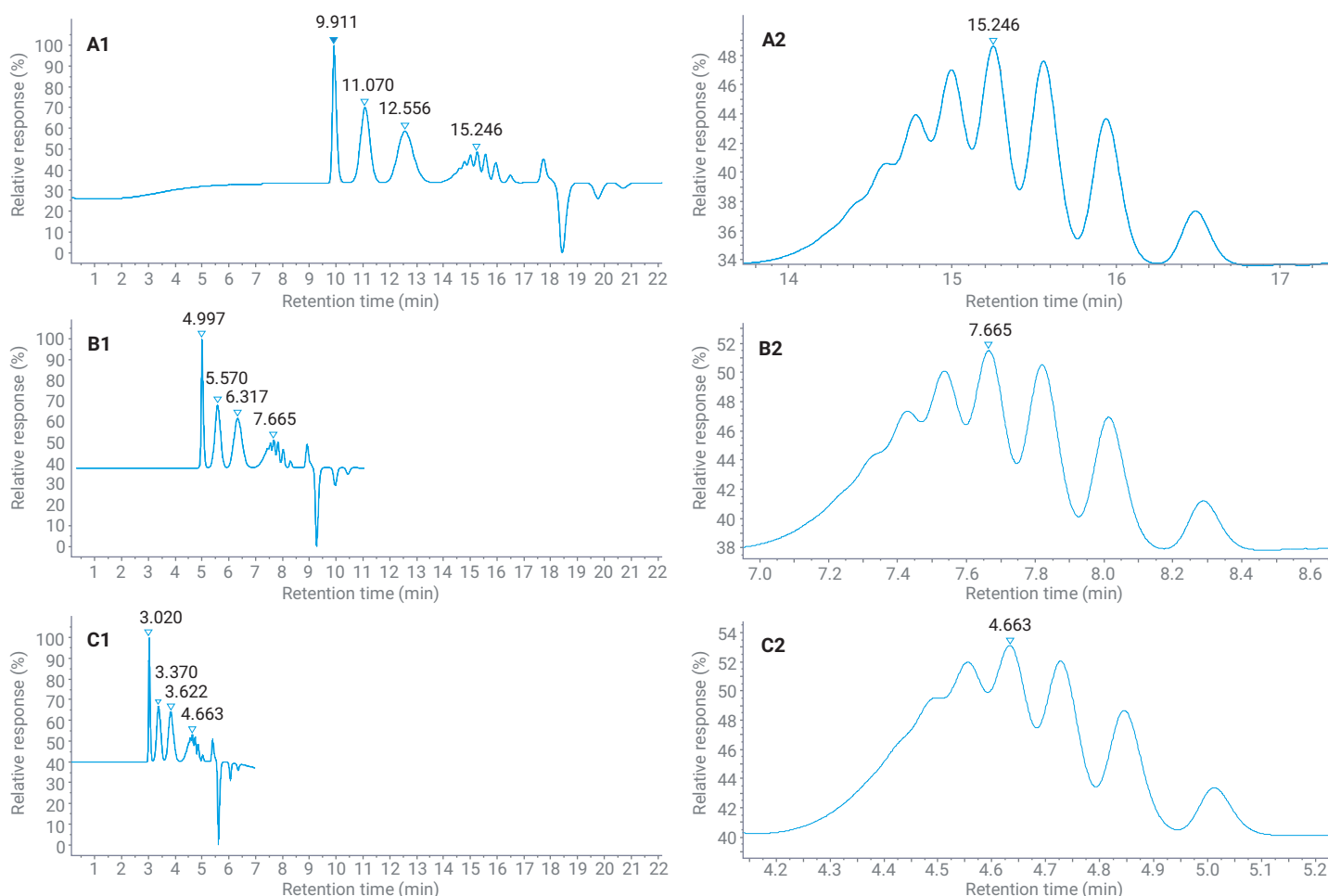


Figure 3. Comparison of the separation of a low molecular weight polystyrene mixture on an Agilent PLgel column (two 250 × 4.6 mm, 3 μm) at different flow rates (A) 0.3, (B) 0.6, (C) 1.0 mL/min THF) and detection with an Agilent 1290 Infinity II Micro RID.

To prove that identical molecular weight results could be obtained at different flow rates, the molecular weight (Mp, Mw, Mn, and PD) of an epoxy resin was determined (Figure 4). The molecular weight distributions (Figures 4A, 4B, and 4C) all show good resolution, with some degradation of the larger oligomers at the highest flow rate. However, the results of the calculated molecular weights are nearly identical (Table in Figure 4).

Table 1. Comparison of solvent consumption per sample and sample throughput of the method on a smaller id column (two 250 × 4.6 mm at 0.3, 0.6, and 1.0 mL/min) compared to the standard method*.

Flow Rate (mL/min)	Run Time (min)	Solvent Consumption Per Sample (%)*	Sample Throughput Factor*
0.3	22	30.0	1
0.6	11	30.0	2
1.0	7	31.8	3

* In comparison to the standard method, 1 mL/min, 22 minute run time, see Figure 1.

Flow Rate (mL/min)	RT (min)	Mp (g/mol)	Mn (g/mol)	Mw (g/mol)	PD
0.3	11.678	7261	2614	6415	2.45
0.6	5.876	7278	2638	6340	2.40
1.0	3.544	7297	2606	6238	2.39

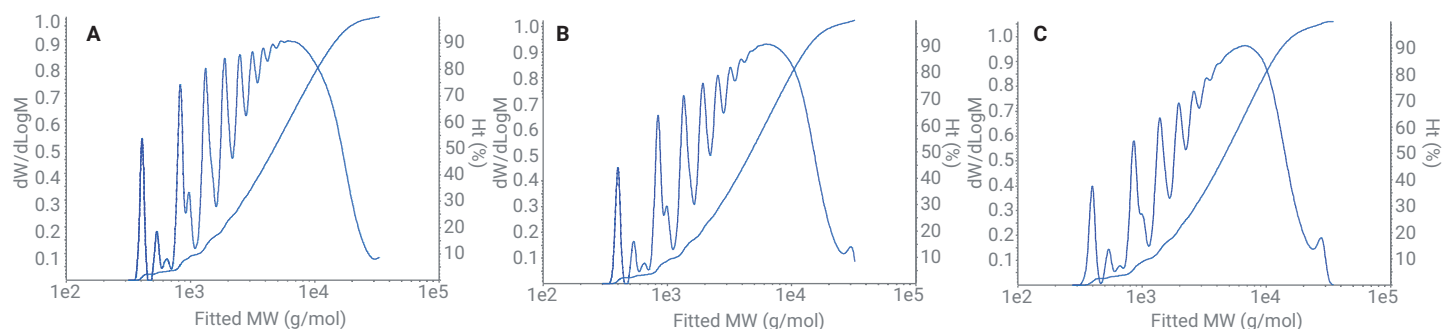


Figure 4. Comparison of the molecular weight of an epoxy resin on an Agilent PLgel column (two 250 × 4.6 mm, 3 μm) at different flow rates (A) 0.3, B) 0.6, C) 1.0 mL/min THF) and detection with an Agilent 1290 Infinity II Micro RID (Mn: number average molecular weight, Mw: weight average molecular weight, Mp: molecular weight at peak maximum, PD: polydispersity index).

The use of the Micro RID in the 1290 Infinity II GPC/SEC system enables the use of narrow-bore GPC/SEC columns with 2.1 mm id for microscale analysis. The downwards calculation of the applied flow rate from the previously shown 4.6 mm id column to the 2.1 mm id column resulted in a flow rate of 60 $\mu\text{L}/\text{min}$. The 1290 Infinity II High-Speed Pump enabled the required flow accuracy to maintain retention times compared to the larger id columns. Another advantage is the lower dispersion volume of the Micro RID cell (2 μL) compared to the standard RID (8 μL), which provides enhanced resolution for lower molecular weight oligomers (Figure 5).

Conclusion

This Application Note demonstrates the use of the Agilent 1290 Infinity II GPC/SEC system with the Agilent 1290 Infinity II Micro RID for classical as well as micro GPC methods. It shows that the separation of polymer mixtures under typical GPC standard conditions with large id columns and high flow rates is equivalent to that gained with a standard RID. In addition, it is possible to use columns with smaller inner diameters to decrease the solvent consumption per sample, and increase the sample throughput. The application of narrow-bore columns enables the use of micro GPC methods at very low flow rates in the microliter range with consistent molecular weight results and improved resolution with the 1290 Infinity II Micro RID.

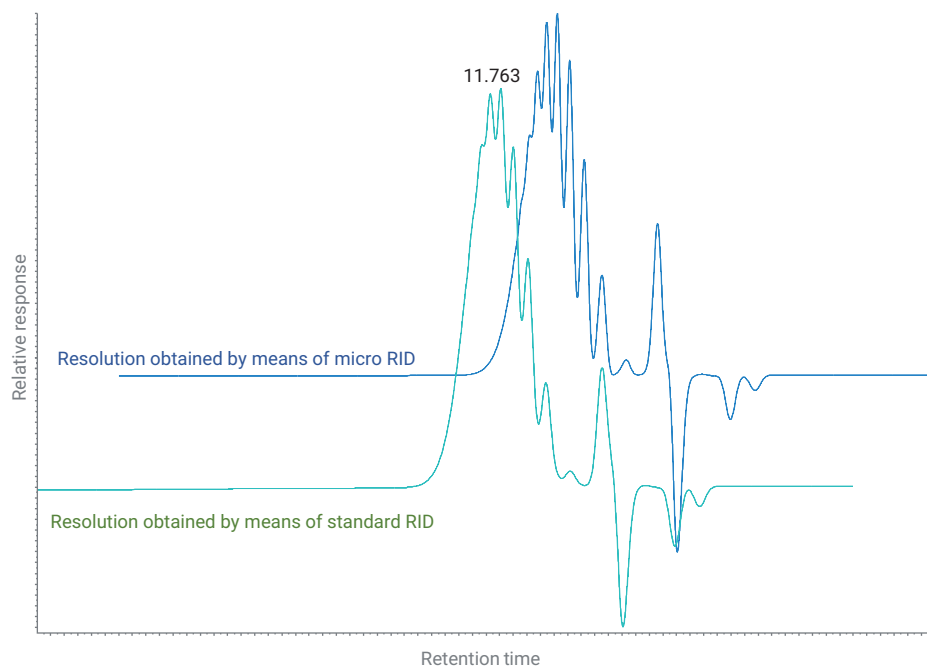


Figure 5. Comparison of the resolution of standard RID versus micro RID for a low molecular weight PS 580 (two Agilent OligoPore columns, 250 \times 2.1 mm, 60 $\mu\text{L}/\text{min}$ flow rate). Due to the stacked display, mode there is no retention time scale shown).

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