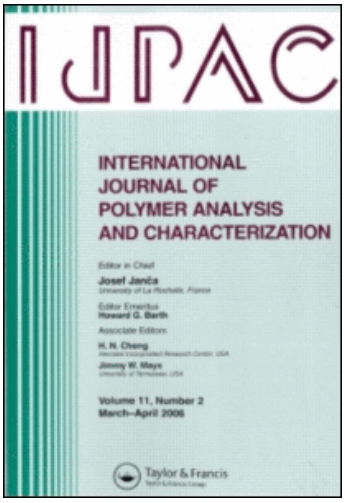

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Intra- and Interday Precision of Molecular Weight Data Determined by GPC: Precision of M_n and M_w

H. Goetz[a]; H. Schulenberg-schell[a]
[a] Pharmaceutical Solutions Business Unit, Agilent Technologies Deutschland GmbH, Waldbronn, Germany

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Intra- and Interday Precision of Molecular Weight Data Determined by GPC: Precision of M_n and M_w

H. Goetz and H. Schulenberg-Schell
Pharmaceutical Solutions Business Unit,
Agilent Technologies Deutschland GmbH,
Waldbronn, Germany

The goal of the investigation was to determine the intra- and interday precision of GPC molecular weight data. Typical GPC conditions with organic eluents were chosen to obtain data close to actual conditions. Good precision data not only improves the reliability of results but also the productivity because less time-consuming recalibrations are needed. The intraday relative standard deviations for M_n and M_w were for below 1.5% with the majority below 1.0%. The interday precision from day 1 to day 20 for M_n was 1.4% and 1.3% for M_w. Retention time stability below 0.06% is required to obtain molecular weight precision data below 1%. Fully automated data acquisition, evaluation, and reporting were found to be more precise in comparison to semiautomated with automated data acquisition and interactive data evaluation.

Keywords: Intra- and interday precision; Molecular weight averages; GPC/SEC

INTRODUCTION

Intra- and interday precision of molecular weight data are important performance aspects of a gel permeation chromatograph (GPC) method.

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Address correspondence to H. Goetz, Pharmaceutical Solutions Business Unit, Agilent Technologies Deutschland GmbH, 76337 Waldbronn, Germany.
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Good intraday precision is needed for obtaining reliable calibration curves for the polymer standards and reliable molecular weight data for the polymer samples. Good interday precision improves the reliability of the data and the productivity of the laboratory because fewer recalibrations are needed. If the instrument is operated in a process-control environment, this can directly influence the productivity of the plant. Daily recalibrations can easily require three hours, as the calculation in Table I shows. If the software cannot update, the calibration curve automatically based on the recalibration runs, an additional half-hour must be added for manually typing the new calibration curve.

The term "precision" is defined in the ISO Standard 5725[1] as the closeness of agreement between independent test results obtained under stipulated conditions. In GPC precision data are obtained by multiple injections of the same sample and are expressed as the relative standard deviation (RSD). The term can be further subdivided into the following:

- Repeatability = Precision under repeatability conditions: Conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time. The relative standard deviation calculated for data obtained within one day is often called intraday (within one day) precision, while that measured over a period of days is termed interday (between days)[2].
- Reproducibility = Precision under reproducibility conditions: Conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment. The intra- and interday precision of molecular weight data obtained by GPC analysis depends on several hardware and software parameters as Table II shows.

Because of its importance and the many influencing parameters, precision is of great interest since the advent of GPC in the late sixties. As reported in the literature, the relative standard deviations in the determination of M_n and M_w varied at that time in the range 3.1 to 6.8%

TABLE I Estimated Time Needed for Recalibration Runs

Assumption	Time (min)
Analysis time for 1 calibration vial with 4 standards (conditions: 3 columns in series, length 300 mm, flow rate 1 mL/min)	30
Analysis time for 2 runs from 3 calibration vials with in total 12 standards (2 runs/vial to avoid stray points)	180

TABLE II Hardware and Software Parameters Influencing Precision

Hardware parameters	Software parameters
Column stability	Precision of calculation procedures
Flow precision	Precision of baseline setting
Temperature precision	Precision of setting calculation start and end marks
Baseline stability	Number of data points
Injection volume precision	Possibility to use an internal standard correction for flow rate changes

and 1.2 to 5.0%, respectively[3–6]. Recent data were obtained mainly during round-robin tests. Bruessau's[8] 1996 intraday precision data obtained in a European round-robin study are shown in Table III. These results are confirmed by a Japanese round-robin experiment published in 1995[9]. Interday precision data obtained over a series of days are not documented and are rarely found in the literature for a longer period, e.g., 20 days.

The goal of our investigation was to evaluate what is nowadays achieveable concerning not only intraday but also interday repeatability of the molecular weight averages M_n and M_w under typical GPC conditions. The data were obtained without the use of the internal-standard flow-correction feature of the GPC data analysis software, because we were mainly interested in the hardware performance of the GPC system.

EXPERIMENTAL

Samples were technical, broad distributed styrene acrylonitrile copolymer and polystyrene, both dissolved in the respective mobile phase, conc. 0.1% w/w. Standards were polystyrene (PS) and poly(methyl methylacrylate) (PMMA), ReadyCal standards from Polymer Standards Service, Mainz, Germany. Columns were 3 × PLGel mixed B in series,

TABLE III Intraday Precision Results of a European Round-Robin Test[8]

Result	Intraday precision (RSD)
M_n	2.5
M_w	1.4
D	2.8

7.8 × 300 mm, 10 μm from Agilent Technologies (part number 79911GP-MXC). Eluents were tetrahydrofuran (HPLC grade), toluene (HPLC grade), and dimethylacetamide (reagent grade) containing 0.05M LiBr.

The flow rate was 1 mL/min. The column compartment temperature with THF was ambient, with toluene: 40°C, and with dimethylacetamide: 70°C. The instrument was an Agilent Technologies 1100 Series GPC System, which includes a vacuum degasser, isocratic pump, autosampler, thermostatted column compartment, refractive index, and diode array detector. For the majority of the experiments, the refractive index detector was used. Instrument control and data analysis was with an Agilent Technologies HPLC ChemStation with GPC data analysis and ChemStore software; the enhanced integrator was used.

RESULTS

Retention Time Precision

First we studied the precision of the retention times, which must be repeatable to obtain good precision of molecular weight data. Because of the lin-log calibration in GPC and linear retention time or elution volume versus the logarithm of molecular weight, retention time precision is of significantly more influence than in the other modes of HPLC.

Intraday Precision of Retention Times of Polymer Standards

A fundamental prerequisite is good intraday precision of the retention times of the polymer standards used for the calibration. Figure 1 shows an overlay of 10 consecutive, automatic injections of a poly(methyl methacrylate) (PMMA) solution consisting of four different standards. Good precision of retention times and peak areas is demonstrated by the fact that it is difficult to visually distinguish between the runs, which is of course a question of enlargement. It is without any doubt demonstrated by relative standard deviations smaller than 0.035% for the retention times.

The calibration curve needs to be updated to take retention time changes into account. The frequency of this update depends on hard- and software performance and application and environmental factors, which are different from lab to lab. With good interday precision, less recalibration is necessary, which improves productivity as described in the introduction. For the present investigation, we recalibrated the system only after a change of the analysis parameters, for example a mobile phase or a column temperature change. Thus calibration was kept constant approximately for one month.

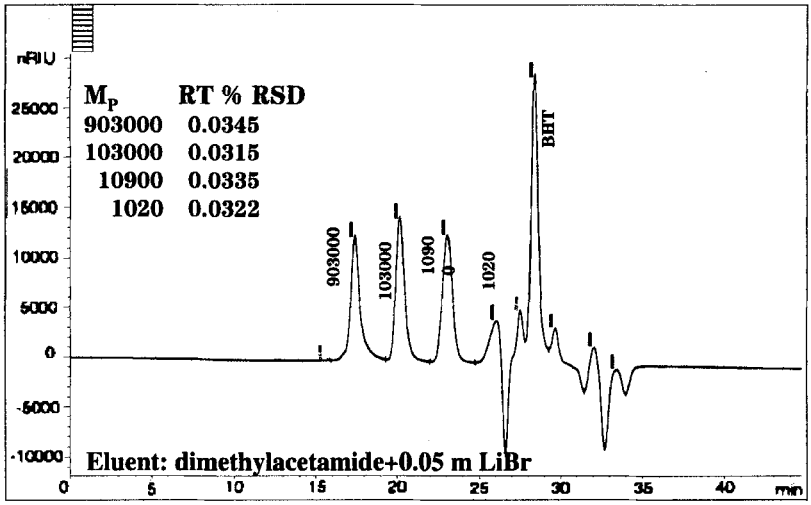

FIGURE 1 Overlay of ten consecutive analyses of a PMMA solution containing four standards.

Intra- and Interday Precision of Retention Times of Polymer Samples and Flow Markers

To measure the precision of the retention times, we injected several technical polymers using the eluents as mentioned in the experimental section. The samples were injected automatically every day in a sequence of at least 20 days. Figure 2 shows the results obtained for the technical styrene-acrylonitrile (SAN) polymer obtained with tetrahydrofuran (THF) as mobile phase. The very good interday precision from the 1st to the 20th day was 0.069%. The intraday precision was always below 0.05% with the exception of days 1, 2, and 15, but still below 0.08% (refer to Table IV). The lower, but still very good, retention time precision is caused by small pressure changes. A small air bubble or a partial and short-term blockage of a frit can cause these pressure changes. The pressure effects are larger in days 1 and 2 since we did not automatically recycle the eluent after each analysis during these days. With "recycle on," the eluent is automatically directed back to the solvent bottle after each analysis; thus eluent is saved and, more important, better conditioned.

Figure 3 shows that a similar curve was measured for the flow marker butylated hydroxytoluene (BHT) and toluene as eluent; the "recycle after analysis" possibility was activated for all runs. The interday retention

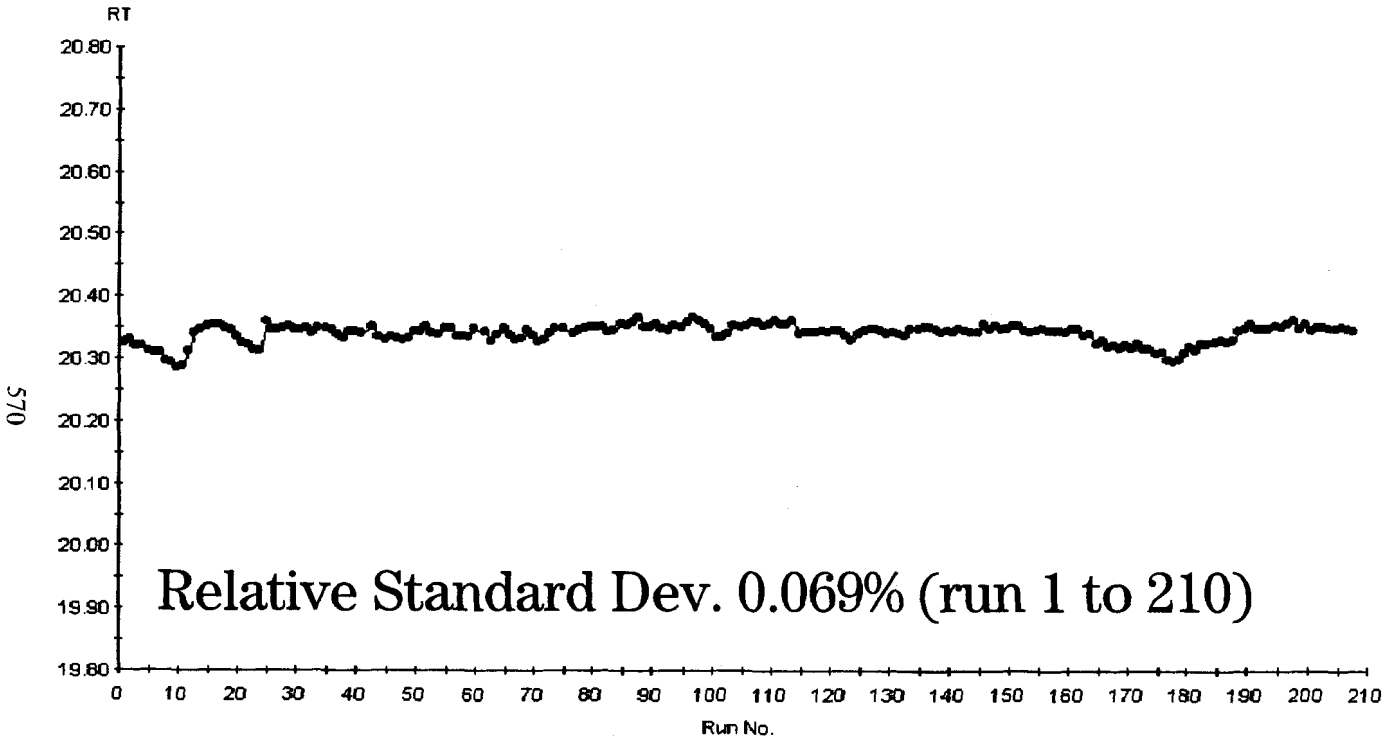

FIGURE 2 Intra- and interday precision of retention times for a styrene-acrylonitrile copolymer (SAN) over 20 days, eluent: THF.

TABLE IV Intra- and Interday Precision of Retention Times, M_n, and M_w for Days 1 to 20

Day no.	%RSD ret. time	% RSD for M_n	%RSD for M_w
1	0.071	1.16	1.12
2	0.075	1.43	0.78
3	0.020	0.92	0.72
4	0.032	0.82	0.83
5	0.030	1.18	0.97
6	0.038	0.95	0.78
7	0.037	1.13	1.08
8	0.030	0.58	0.81
9	0.043	0.91	0.66
10	0.025	0.73	0.32
11	0.022	1.43	0.43
12	0.021	0.81	0.35
13	0.016	0.89	0.59
14	0.029	0.88	1.19
15	0.065	1.08	1.27
16	0.002	0.68	0.70
17	0.045	0.99	0.85
18	0.038	0.94	0.78
19	0.041	0.95	0.80
20	0.009	0.70	0.71
Av. %RSD	0.035	0.96	0.78

time precision for all 280 runs was 0.063%, and all intraday precision data were below 0.075%.

Precision of Molecular Weight Data of Polymer Samples

Intraday Precision

After the intra- and interday precision of the retention times was shown, we determined the molecular weight data repeatability for various polymers. Figure 4 is an overlay of ten consecutive analyses of the styrene-acrylonitrile copolymer as obtained on day 1. The relative standard deviations of retention times were below 0.06% and of M_n and M_w below 1.2%. It should be pointed out that these and the following data were obtained using an automatic baseline setting for a broad sample with a polydispersity D of 2.10 and not for a narrow distributed polymer

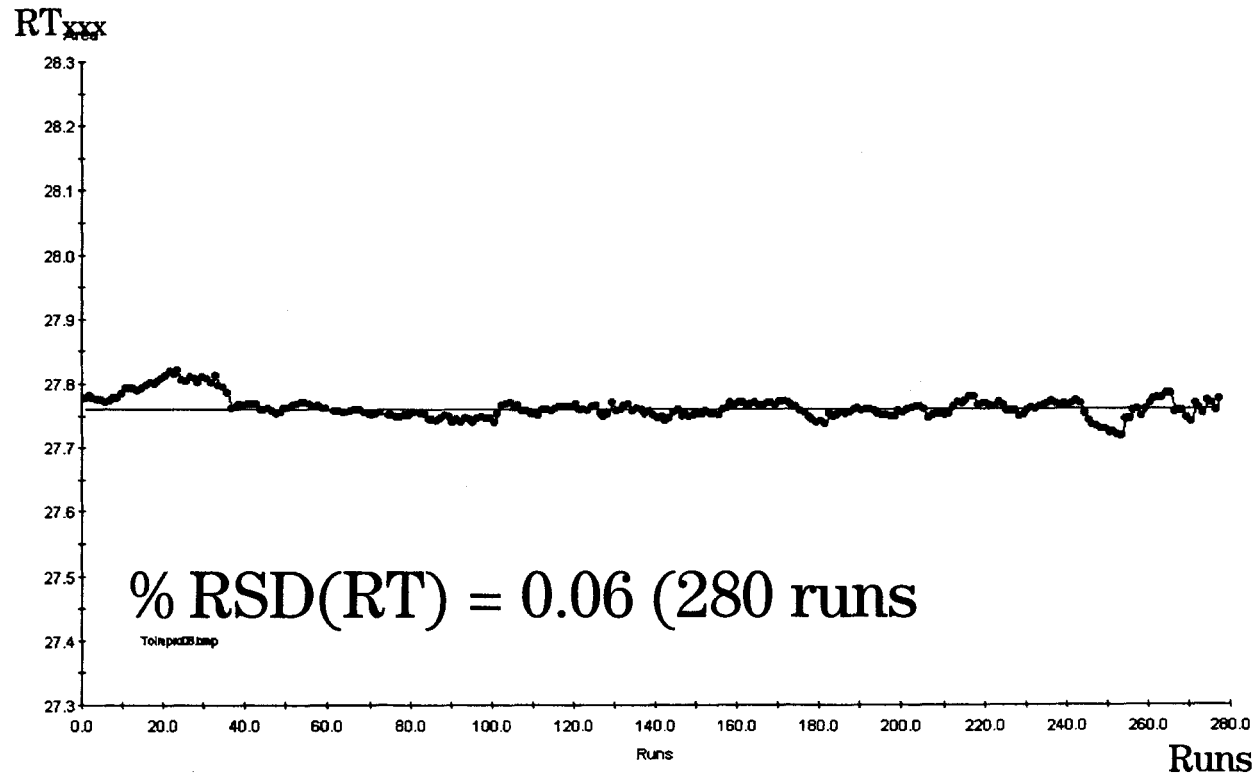

FIGURE 3 Intra- and interday precision of retention times of butylated hydroxytoluene over 20 days/280 injections, eluent: toluene.

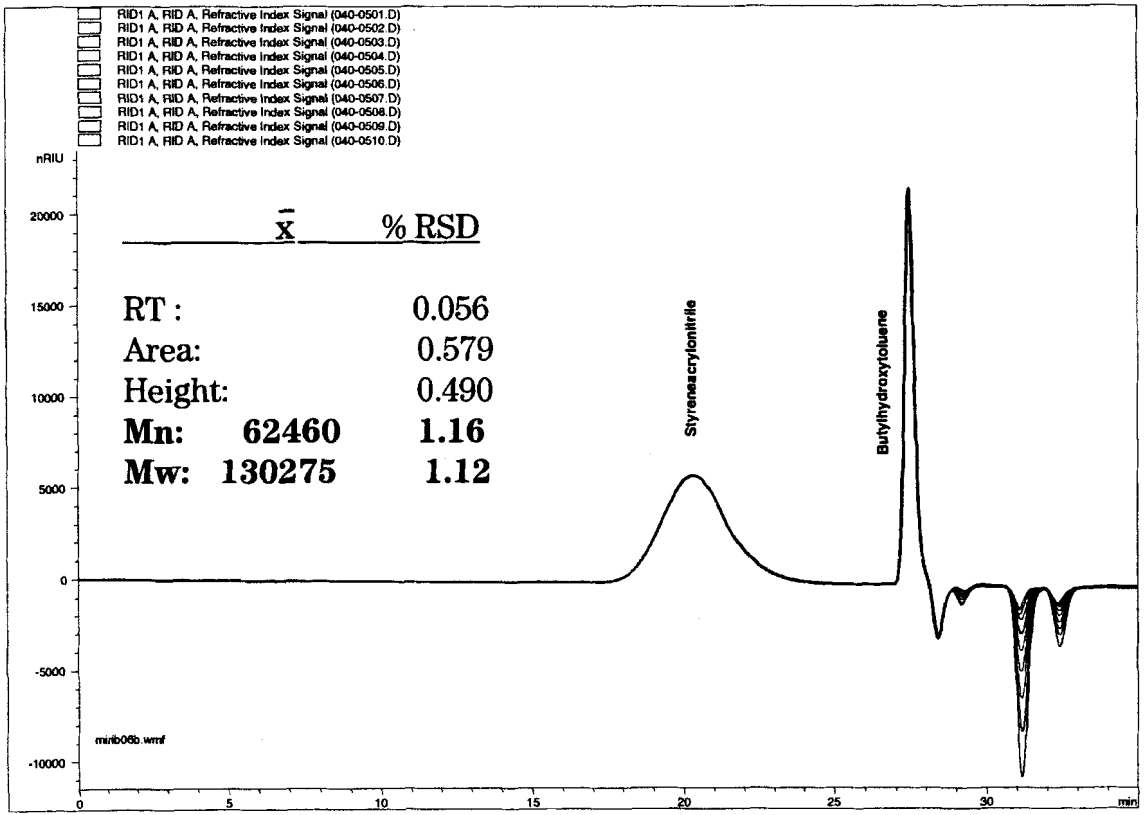

FIGURE 4 Overlay of ten automatic runs of a polystyrene-acrylonitrile copolymer and intraday precision on day 1. Eluent: THF, other parameters as described in the experimental section.

standard. In the latter case, it is significantly easier for the software to determine the start and end marks of the peak, which have a significant impact on the results. Figures 5 and 6 show the results obtained for days 10 and 20, which are even better.

Table IV shows the M_n and M_w relative standard deviations calculated for each of the 20 days (intraday precision). We see that they were always smaller than 1.50% with an average value for M_n of 0.96% and of M_w of 0.78%. Table IV further shows that a retention time precision of less than 0.06% is typically required to obtain relative standard deviations for the molecular weight data below 1%.

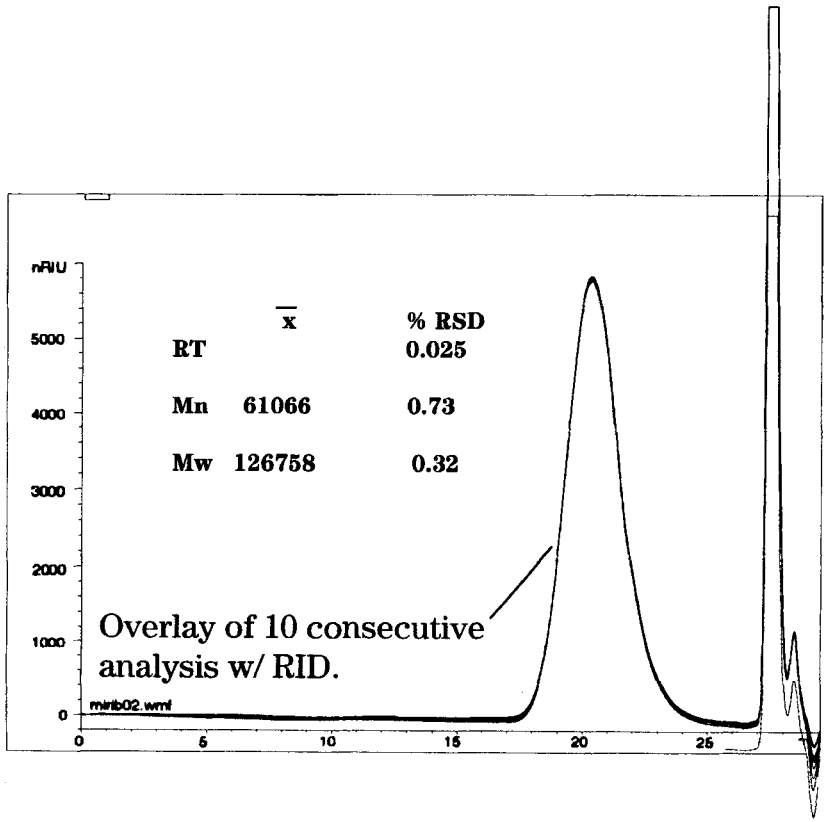

FIGURE 5 Enlargement of the chromatograms obtained on day 10 and precision data for this day. Eluent: THF, other parameters as described in experimental section.

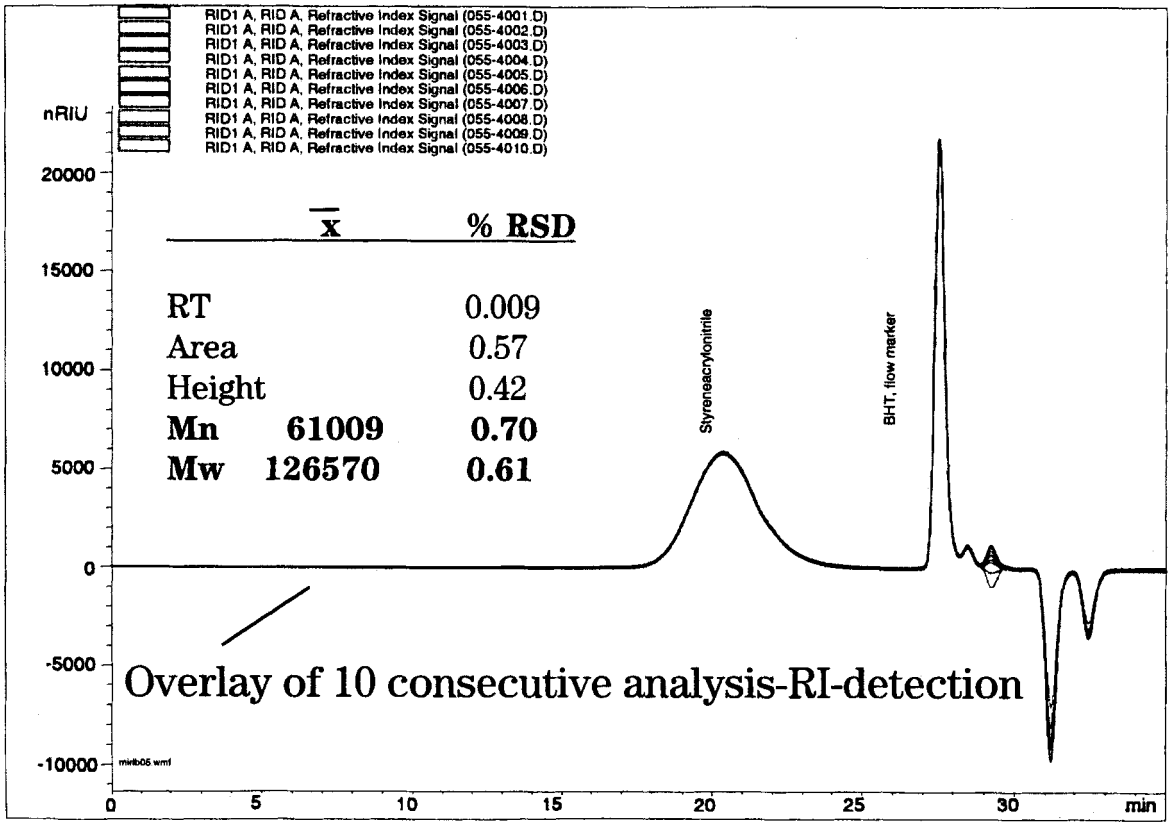

FIGURE 6 Overlay of 10 automatic runs of a technical polystyreneacrylonitrile polymer and intraday precision on day 20. Eluent: THF, other parameters as described in the experimental section.

Interday Precision

Good interday precision is important to ensure that polymer samples, e.g., from different production days, can be characterized with sufficient confidence and without the need of daily, time-consuming recalibration. Figure 7 gives a first visual overview on the interday precision obtained with the styrene-acrylonitrile copolymer and THF as eluent. It is an overlay of the injections made on days 1, 5, 10, and 20.

Table V shows the calculated relative standard deviations. It should be pointed out that these data take almost all injections from day number 1 until day number 20 into account. Only about 10 injections had to be filtered out. They were stray points, e.g, caused by an incorrectly filled vial. The maximum single-day deviations from the average values for M_n and M_w were respectively 3.6 and 2.5% (refer to Table V).

Automatic vs. Interactive Evaluation

It is widely accepted among polymer analysts that the data acquisition part of the analysis can be automated with modern, state-of-the-art GPC hardware without any loss of accuracy and precision. Concerning data analysis, there is still discussion whether automatic or interactive baseline

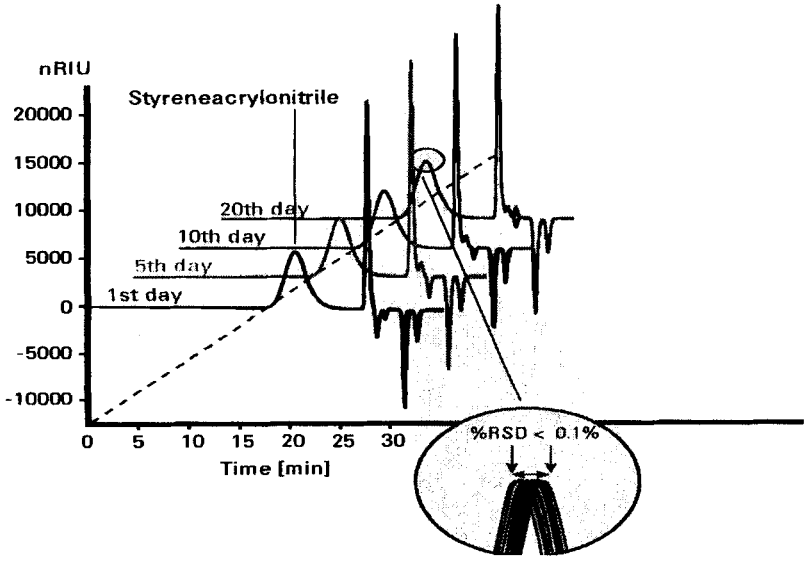

FIGURE 7 Overlay of all analyses of the styrene-acrylonitrile copolymer on days 1, 5, 10, and 20. The image in the bottom is an enlargement of the analyses on day 20.

TABLE V Interday (1st to 20th day) Precision as Determined for All Runs of Poly(styrene-acrylonitrile). Eluent THF, Other Parameters as Specified in the Experimental Section

Result	Relative standard deviation (RSD)	Average value	Maximum deviation from average value for a single run
M_n	1.41	61000	3.6%
M_w	1.32	127700	2.5%

setting should be used. To study this effect, we have analyzed a technical polystyrene sample ten times with interactive and another ten times with fully automatic data evaluation. Figure 8 shows an overlay of the ten chromatograms for the technical polystyrene sample and the intraday precision data for automatic and interactive baseline setting. We see that the precision data for the automatic mode are slightly better than for the interactive mode for both M_n and M_w. We found similar improvement also for the other polymers analyzed for this article. This will be not the case for every sample, e.g., for a polymer with a strongly tailing peak or when the peak height is small due to low sample concentration or low refractive index.

In most cases the ChemStation "Enhanced Integrator" is perfectly suited to detect the start and the end of a polymer peak correctly and to ensure reliable automation. It provides optimized baseline tracking using parameters from the individual method and data files and better peak allocation. It also has additional initial parameters to remove noise-generated peaks through the initial height parameter, and for ease of use—the "enhanced integrator" algorithm has a new user interface based on tool bars and automatically focuses on key information. For a detailed discussion of the integration algorithm refer to Ref. [10].

Typical advantages of completely automated analysis (from data acquisition to reporting) are that it offers at least similar precision, less room for human interpretation and errors, higher traceability and consistency, and frees trained personal from time-consuming work thus improving efficiency.

Refractive Index vs. UV Detection

All analyses discussed so far were obtained with refractive index detection. This detector is most frequently used for polymer characterization by GPC because many polymers do not absorb in the UV/Vis range, and the refractive index is independent of the molecular weight (starting with a certain minimum molecular weight). However, quite a

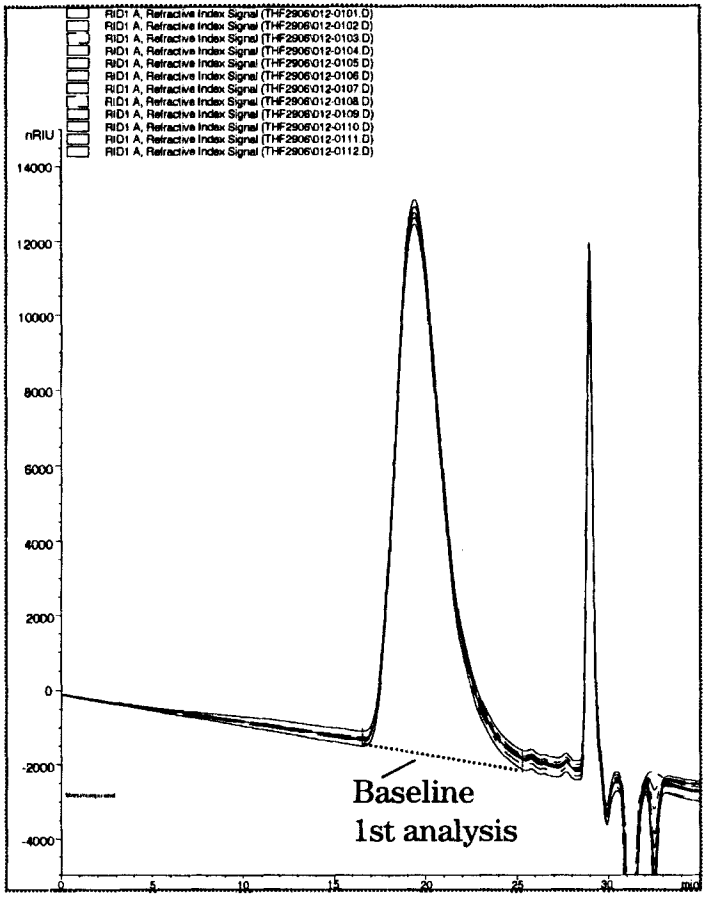

FIGURE 8 Polystyrene sample analyzed with interactive and automatic baseline setting/evaluation. M_n %RSD was 0.92 and 0.57 for interactive and automatic settings, respectively; M_w %RSD was 0.44 and 0.35 for interactive and automatic settings, respectively.

number of polymers can be analyzed with UV/Vis detection. Figure 9 shows a comparison of the intraday precision between RI and UV detection of a broad distributed polystyrene sample obtained from ten analyses. Although modern, state-of-the-art refractive detection has significantly improved in terms of baseline noise and drift, and automation capabilities, there is still some difference as shown in the repeatability data in the table within Figure 9. In our experience the precision data for UV/Vis detection are typically better by a factor of two.

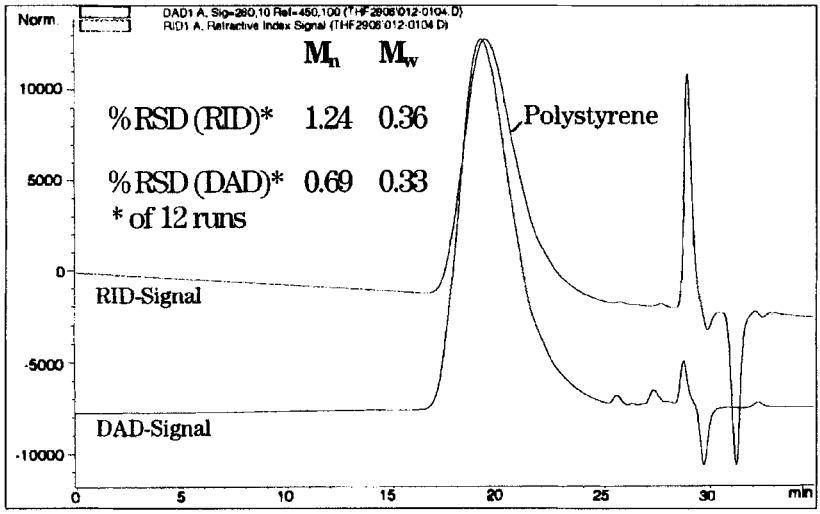

FIGURE 9 Comparison of intraday precision between RI and UV detection.

CONCLUSIONS

The intra- and interday precision of M_n and M_w molecular weight data obtained by GPC has increased significantly in recent years. Using state-of-the-art equipment intra-(within one day) and interday precision data (over 20 days) for M_n and M_w below 1.5% were calculated in completely automated analyses for broad distributed polymers with THF as eluent. The main contributions to these results come from HPLC pumps with an intra- and interday flow stability better than 0.1% (based on polymer retention time), and column thermostats with a temperature precision better than 0.5°C. The use of eluent recycling after the analysis leads to a better conditioned system. Refractive index detectors with low noise (2.5×10^{-9} RIU)[11] and low drift (200×10^{-9} RIU/h)[11] for correct and repeatable baseline and integration window settings are used. Furthermore, software with flexible and repeatable integration and calculation algorithms to adapt to broad polymer peaks and full automation capabilities to reduce human errors is available.

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