Note C1: Faster LC

Introduction

Many chromatographic separations that require peaks to be resolved for analysis take a considerable time to complete. It may also be necessary to repeat an analysis many times so that an estimate of the errors may be obtained. Therefore, an analysis with a run time of 1 hour can take a day. By using data reconstruction techniques, the resolution can be recovered for very short run times and the results are fully quantified.

Data

This example illustrates the potential time saving through using data reconstruction methods. The data are a standard mixture of 15 components used at a laboratory to test their LC column performance. The normal run time is 1 hour but this was reduced to 8 minutes to produce data with severely overlapped peaks so that the ***ReSpect™*** deconvolution program (***Sleuth™***)could be evaluated. Three runs were made with a flushing run between each. This reduced the total experiment run time from 7 hours to less than 1 hour.

Methodology

The main PPL deconvolution program uses a single model and the best results will be obtained only if the applied model matches the profile of all the peaks in the data. Since the peak width may change across the data in most LC experiments it is necessary to either take this into account during the deconvolution by using a model that varies with elution time or pre-process the data so that all peaks have a similar width, allowing a single model to be used. Rescaling of the retention time axis was used for the data presented in this example. This was achieved by estimating the peak width at the beginning and the end of each run and then applying a linear interpolation. Each file was then baseline corrected and deconvolved using a model that fitted the last peak. The rescaling may be reversed so that results are presented on the original scale.

Note that rescaling data is no longer used and a variable model is preferred. This is because information can be lost in the rescaling process. At the time of this work the variable modeling facility was not incorporated into the deconvolution interface.

Results and Discussion

The baseline corrected data for the three experiments are shown in the Figure 1 below. The observed small differences between the files are typical of the variation expected for repeat experiments.

Figure 2 shows the deconvolved results. All deconvolutions are well resolved and all 15 components are clearly separated from their neighbours. The fully quantified results are shown in the table. The total intensity for each file has been normalised to 100% to eliminate differences in injection volume. The quoted error bars are for 99% confidence (3 standard deviations).

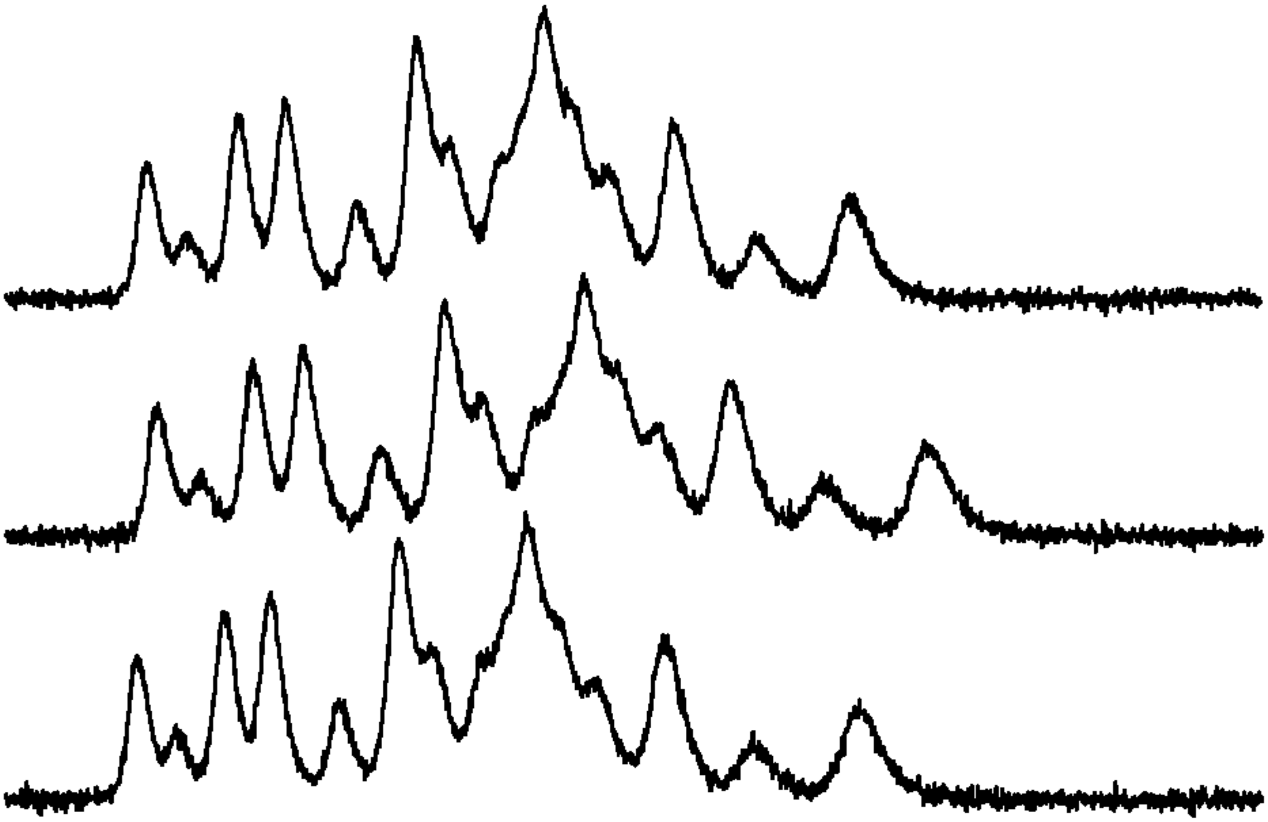

Figure 1. Baseline corrected raw data. Top: Run 1; Centre: Run 2; Bottom: Run3

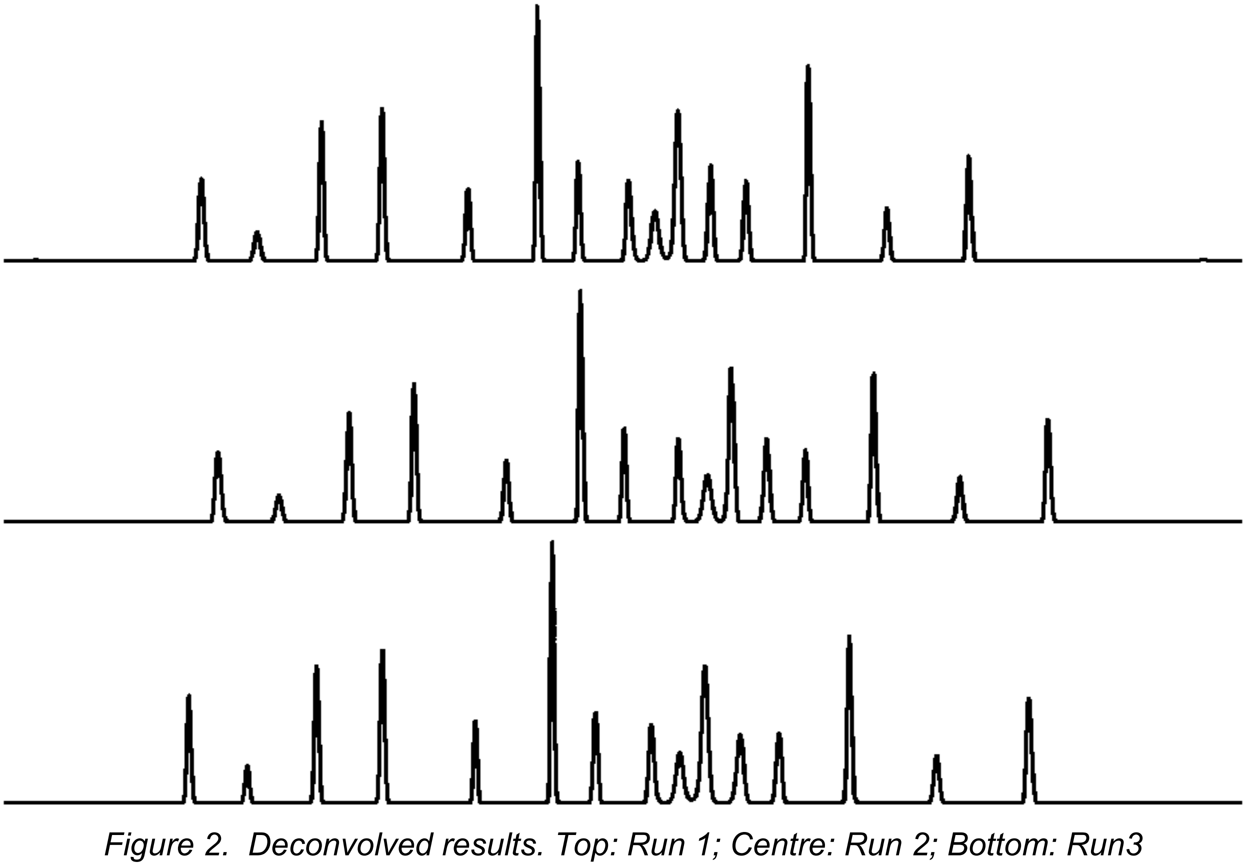

Figure 2. Deconvolved results. Top: Run 1; Centre: Run 2; Bottom: Run3

Note that the three corresponding deconvolved results in Figure 2 are shown on the rescaled axis.

Quantified Results

Peak	Run 1	Run 2	Run 3
1	5.56± 0.04	5.59± 0.06	5.72± 0.06
2	2.29± 0.08	2.23± 0.07	2.13± 0.08
3	7.76± 0.06	7.72± 0.07	7.69± 0.07
4	8.51± 0.06	8.59± 0.06	8.64± 0.07
5	4.21± 0.07	4.27± 0.08	4.13± 0.08
6	12.32± 0.07	12.24± 0.07	12.37± 0.08
7	5.56± 0.10	5.55± 0.10	5.47± 0.10
8	5.77± 0.11	5.70± 0.11	5.53± 0.12
9	5.18± 0.22	5.33± 0.23	5.20± 0.24
10	11.91± 0.20	11.75± 0.16	12.02± 0.18
11	6.31± 0.13	6.31± 0.10	6.39± 0.11
12	5.02± 0.10	4.82± 0.10	4.91± 0.09
13	9.51± 0.07	9.59± 0.07	9.58± 0.08
14	3.39± 0.07	3.49± 0.09	3.38± 0.08
15	6.71± 0.06	6.81± 0.07	6.85± 0.07

An examination of the quantified results shows that, apart from Peak 1, the intensities of the peaks agree within the computed error bars. This is within statistical expectation. In fact, any one of these results would be sufficient to provide a robust analysis but it is recognised that regulatory authorities would insist on more than a single result. The total data processing and subsequent analysis takes just a few minutes.

Conclusions

This example demonstrates the potential for reducing the experimentation time needed to obtain high quality, fully quantified results. In this case the time for the analysis was reduced from 7 hours to less than 1 hour.

Additional experiments not described here show the ***Sleuth*™** results to be more consistent than those from the standard analysis. This is due to errors arising from the way intensities are normally measured. The standard procedure averages the intensities determined from valley to valley and tangential skimming methods, both of which are known to be unreliable.