Positive Probability Ltd

Note C2: LC – Improving Detection Limits

Introduction

Conventional filters allow peak intensities to be reliably integrated when they are completely separated from other peaks. However, in many analytical problems it is not possible to resolve components completely and a weak peak on the side of an intense one may be masked. Therefore, when peaks overlap it is not generally possible to estimate intensities without first applying some type of deconvolution to improve resolution.

Data and Data Processing

In this example, the sample is a mixture of two optically active isomers that have been partially separated using a chiral stationary phase. The requirement is to analyse the relative amounts of the two isomers. Mixtures of the two pure enantiomers were prepared and separated in sequential runs under identical conditions. The R and S proportions were respectively 100:100, 100:20, 100:5 and 100:1. For each file the baseline was corrected and the model automatically computed from the 100:100 mixture. Each file was then deconvolved using the ***Sleuth™*** program. The baseline corrected data for the four mixtures are shown in Figure 1 below. For clarity, the position and vertical scaling of each file has been adjusted so the R isomer (left hand peak) has the same position and amplitude in each trace.

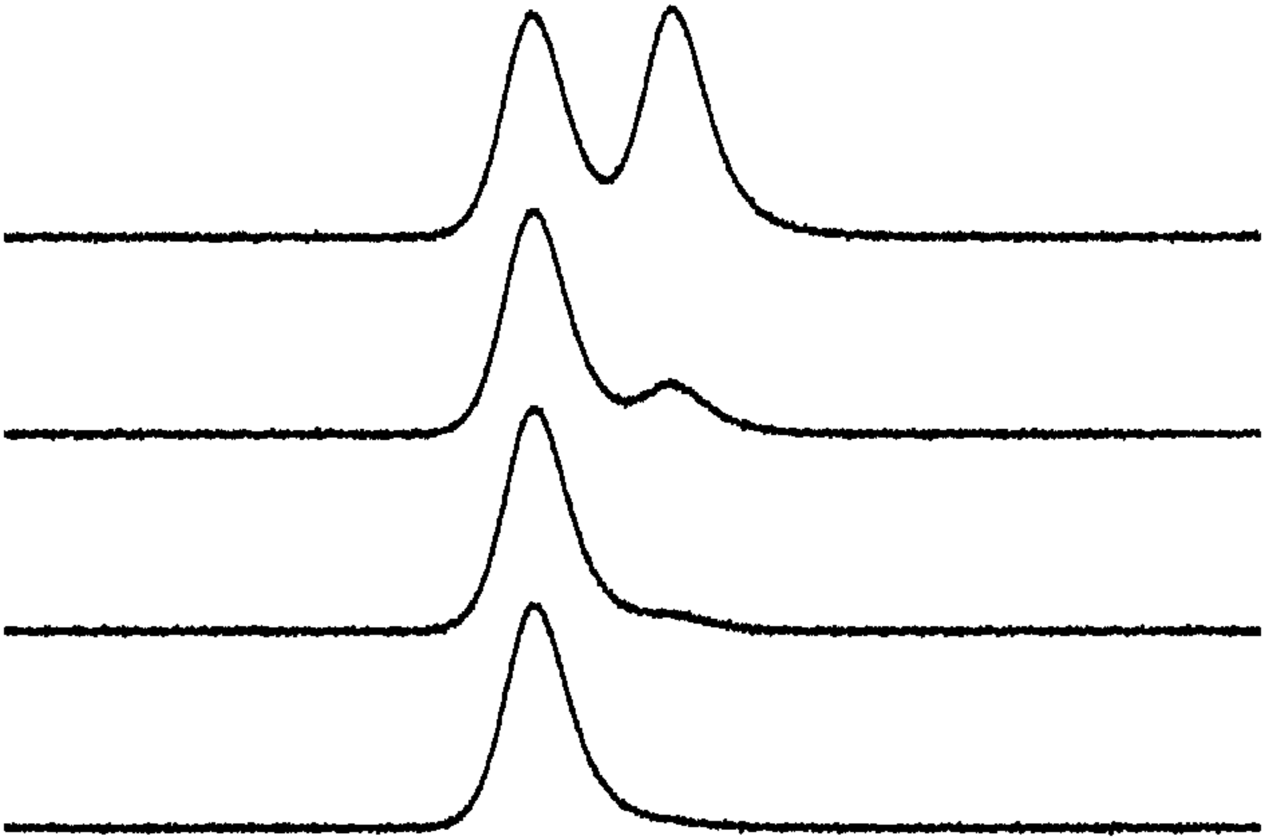

Figure 1. R & S isomer mixtures: Top to bottom: 100:100; 100:20; 100:5; 100:1.

Results and Discussion

Figure 2 below shows the deconvolved results. The traces have been displaced for clarity.

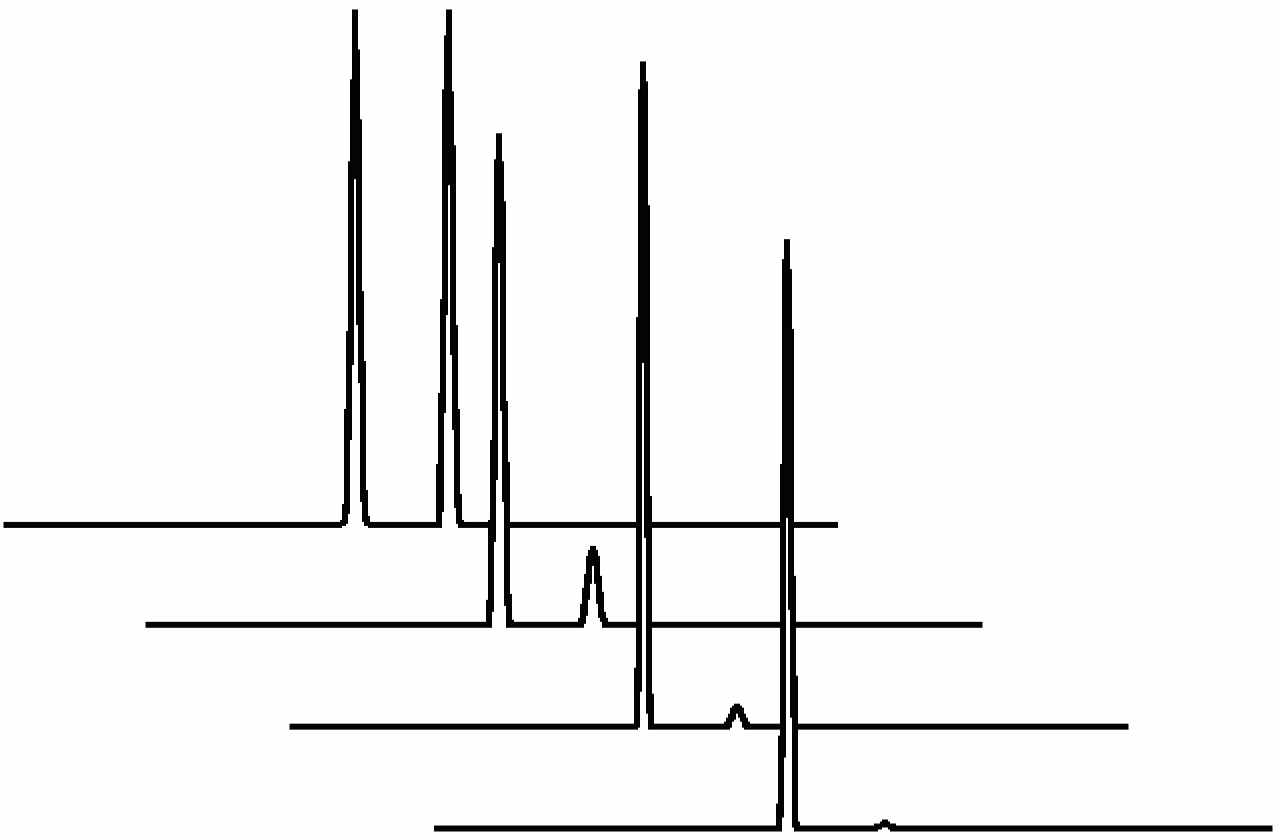

Figure 2. R & S isomer mixtures (Deconvolutions): Top to bottom: 100:100; 100:20; 100:5; 100:1.

For each sample the found proportion of the S isomer was expressed as its percentage intensity of the R isomer to eliminate differences in injection volume. The quantified results are shown in the table below. The quoted error bars are for one standard deviation.

File	% Theory	% Intensity
1	100	100.00± 0.10
	100	99.96± 0.09
2	100	100.00± 0.07
	20	19.79± 0.11
3	100	100.00± 0.06
	5	4.91± 0.12
4	100	100.00± 0.04
	1	0.95± 0.13

From the above table it is clear that even for a 100:1 mixture, the full intensity of the minor component has been recovered within the errors. Three of the determinations fall within the standard error and one (File 2, 20%) is between one and two standard deviations. For only 4 results this is in accord with statistical expectation.

It should be noted that the error for the reference peak (R isomer) falls as the amount of S isomer is reduced, as there is less interference with its signal. At the same time, the certainty of the S isomer intensity falls and its error increases slightly.

Conclusions

This example illustrates the benefits of data reconstruction methods to extract more information from data with overlapped peaks and to provide improved detection limits and quantified results on minor components. It also demonstrates that the ***ReSpect™***–based data reconstruction technology provides quantified error bars that are consistent with statistical expectation.