Note P6: Variable Peak Width Deconvolution – ESI-MS

Introduction

When a deconvolution is required and the peak width in sampling intervals (data points) changes significantly and systematically across the data, the deconvolved result quality will be compromised by using an average model because it will be too wide for some peaks and too narrow for others. Although it is straightforward to use a fully varying model, there is a serious time penalty that would generally be unacceptable. An alternative is to work with segments using a different model for each. With careful design of the methodology, speed gains of up to x10 can be achieved. The example presented here illustrates the benefit of this approach for data in which the data peak width changes markedly.

Data and Data Processing

The data are a 50:50 mixture of normal haemoglobin and a variant in which the β-chains differ by 9.0 Da. The β peaks for each charge are unresolved and a deconvolution is required. The α peaks increase in width by almost x2 with increasing m/z. The data and baseline corrected result are shown in Figure 1.

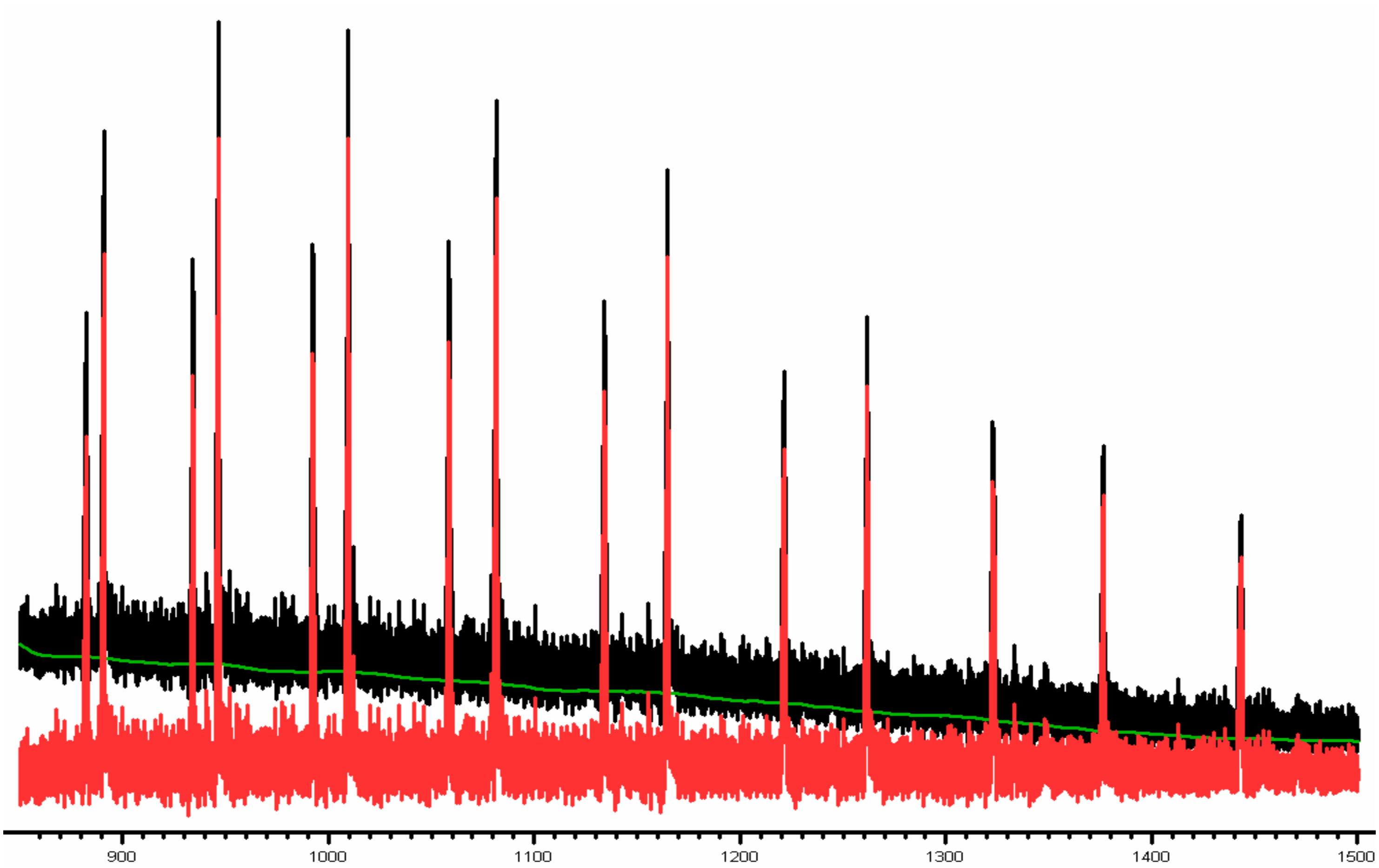

Figure 1. Data (black), computed baseline (green) and baseline corrected result (red).

The α peak at m/z 1165 was used as an average model for a deconvolution and was 26.3 sampling intervals wide. The peaks at high charge are less than 19 sampling intervals wide and so this model is far too wide at the low m/z end of the data. By additionally modelling the highest and lowest charges for the α peaks, the way the model varies is obtained and a second deconvolution may be performed that takes this into account. Both deconvolutions used the ***ReSpect*™**–based ***Sleuth*™** data reconstruction methodology.

Results and Discussion

Figure 2 shows the deconvolved result for the highest two charges using the average model. The β peaks are on the left. Because the model is too wide for this region, the highest charge β peaks are not resolved and the next lowest is poorly resolved.

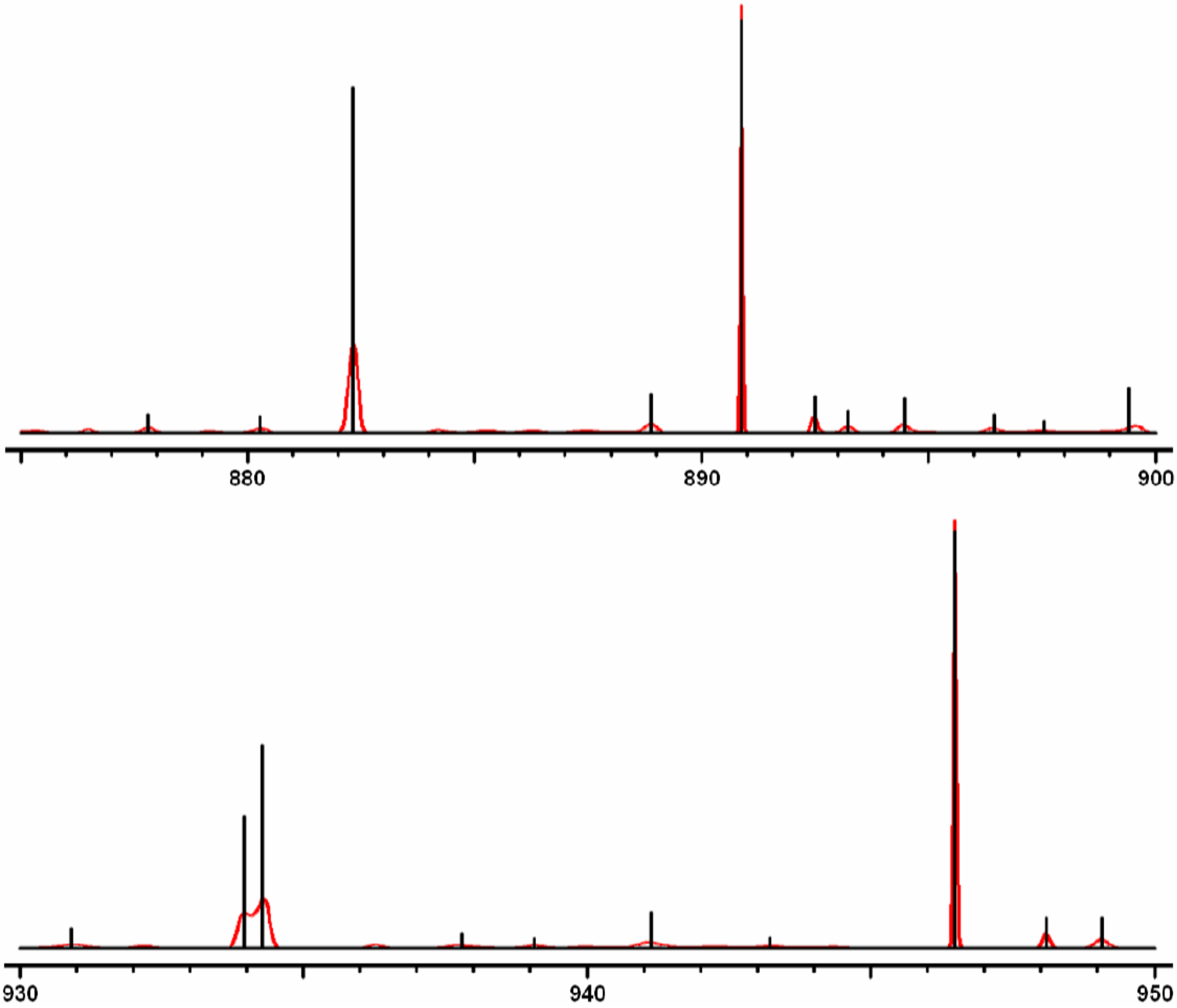

Figure 2. A too wide model does not efficiently resolve the β peaks (spikes are for 1 SD).

Figure 3 shows the result of using a model that varies with the change in peak width. Note that the highest charge β peaks are now cleanly resolved and that the intensity ratio for both charges is close to the expected 1:1.

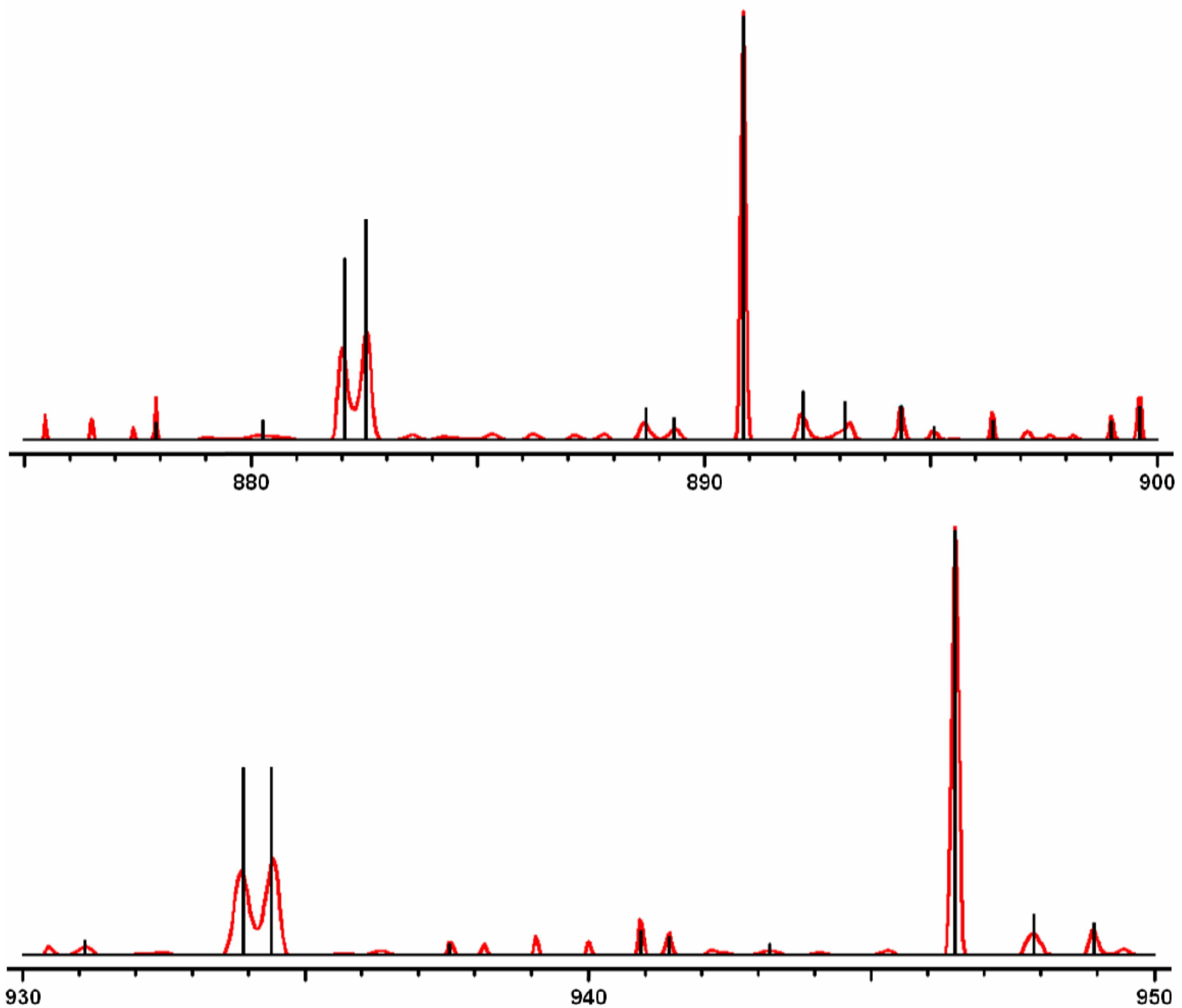

Figure 3. A varying model provides superior resolution for the β peaks (spikes are for 1 SD).

The improved deconvolution resolution provides an improved mass accuracy and more reliable peak intensities. The latter arises from the correct model at any point in the data ensuring that close, overlapped peaks (including Na and K adducts) and noise features are not incorporated into the main peaks, as illustrated in the results table below.

For completeness, a horizontal expansion of the charge deconvolved result is shown in Figure 4.

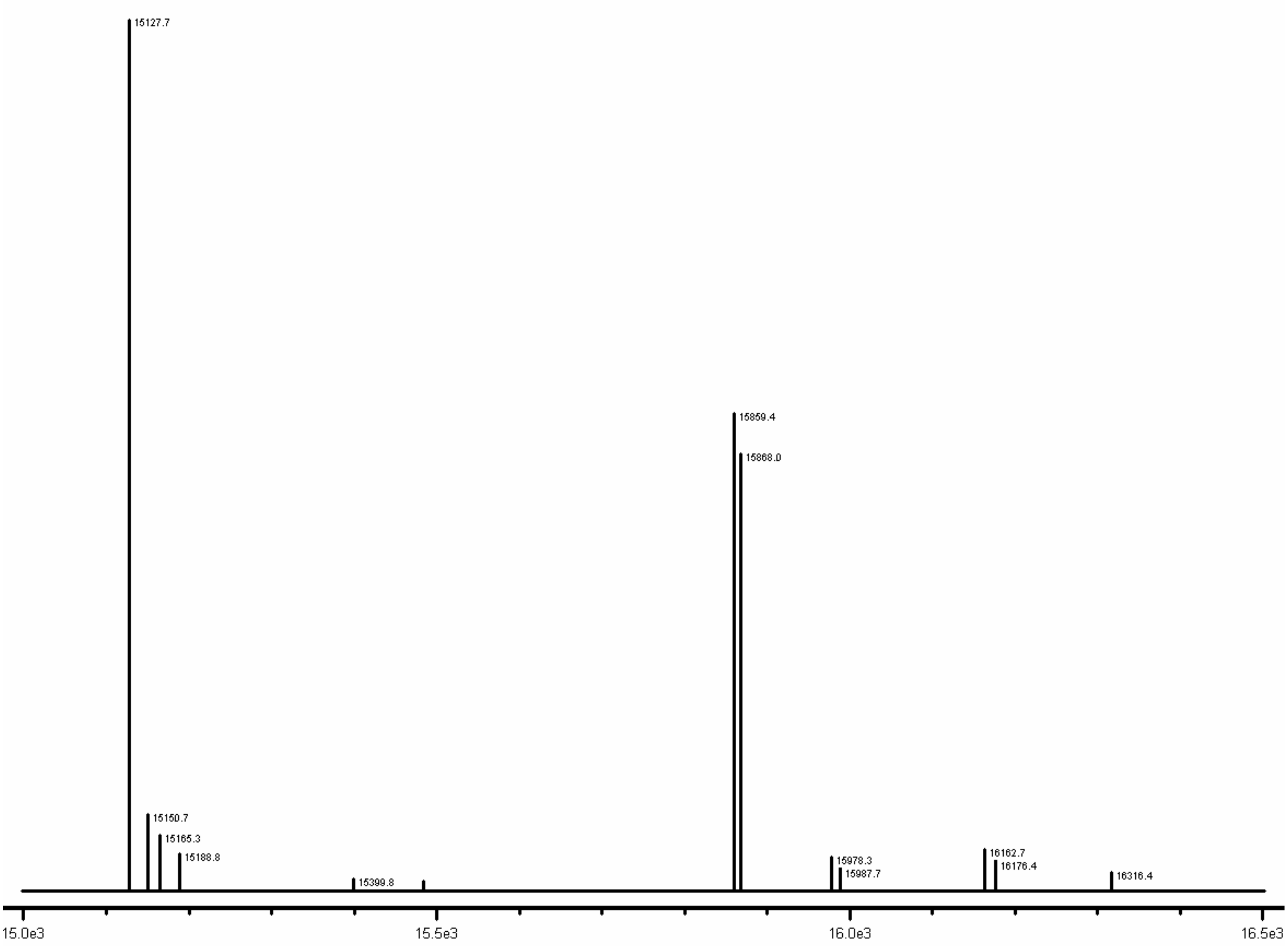

Figure 4. Charge deconvolved result for the relevant masses.

The table below compares the results for the α and β peaks using a fixed and a variable model. Salient points are: Orange highlight shows average mass errors, green highlight the β separation and blue highlight the β intensity ratio.

	Average model						**Varying model**					
	Mass	**M err**	**Intens**	**Dif**	**Dif err**	**Ratio**	**Mass**	**M err**	**Intens**	**Dif**	**Dif err**	**Ratio**
Alpha	15127.64	0.53	2.5E+08				15127.65	0.53	2.4E+08			
+Na	15150.82	0.77	1.9E+07	23.18	0.93		15150.67	0.91	2.1E+07	23.02	1.05	
+K	15167.02	1.79	1.6E+07	39.38	1.87		15166.32	1.24	1.5E+07	38.67	1.35	
+NaK	15189.51	1.49	1.3E+07	61.87	1.58		15188.76	1.50	1.0E+07	61.11	1.59	
Beta1	15859.83	1.61	1.5E+08			**43.4**	15859.36	0.56	1.3E+08			**49.8**
Beta2	15867.31	2.12	1.1E+08	**7.48**	2.66	**56.6**	15867.98	0.56	1.2E+08	**8.62**	0.79	**50.2**
	Ave err	**1.39**					**Ave err**	**0.88**				

In the above table orange highlight shows the average mass errors and that the error is reduced using a variable model. The green highlight shows that the found beta separation is closer to the known value and that the mass errors are much reduced for the variable model deconvolution. The blue highlight shows that the beta intensity ratio is in agreement with the known ration using a variable model.

Conclusions

This example demonstrates that using a variable model for data where the peak width varies provides superior quality results. Salient points to note are:

1. Average mass errors are smaller using a variable model.
2. A variable model provides improved mass accuracy (beta separation).
3. Peak intensities are improved with a variable model (beta intensity ratio).