Rapid evaporative ionisation mass spectrometry analysis of fish mince samples

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Overview and notes

- Rapid evaporative ionisation mass spectrometry (REIMS) is a technique that allows rapid high resolution mass spectral fingerprinting of samples with no sample preparation
  - High resolution masses can be used to do basic compound identification
  - Possible to do MS/MS on selected features but a bit time consuming
- Fish mince samples
  - Samples prepared and provided by Plant and Food Research: 95 samples, 9 QCs, all prepared in triplicate across 15 x 24 well culture plates (306 samples in total – possible mismatch between plates and plate map for Plate 5)
- Samples analysed using REIMS in negative ionisation mode, taken straight from -80 C
  - Likely improvement if samples were thawed slightly beforehand – frosting interfered a bit with laser signal
  - About 8 minutes per plate, though not including time required to clean the source and laser head, nor to calibrate the instrument (half a day, once a week or before a project)
  - 2080 features detected after basic processing, 1384 features after QC-based feature reduction (see below)
- Initial data analysis
  - Data were explored using unsupervised (PCA) and supervised methods (OPLS-DA) for the comparison between ‘H’, ‘M’ and ‘HM’
  - ‘QC reduced’ data set also analysed – all features with an RSD >30 % in the QC sample were removed. Similar results to full dataset but fewer features. Useful for removing noise.
    - For measurements over an extended period of time, QCs become very important
    - No use of the QC samples for drift correction in this case, though this would probably reduce analysis-related variation

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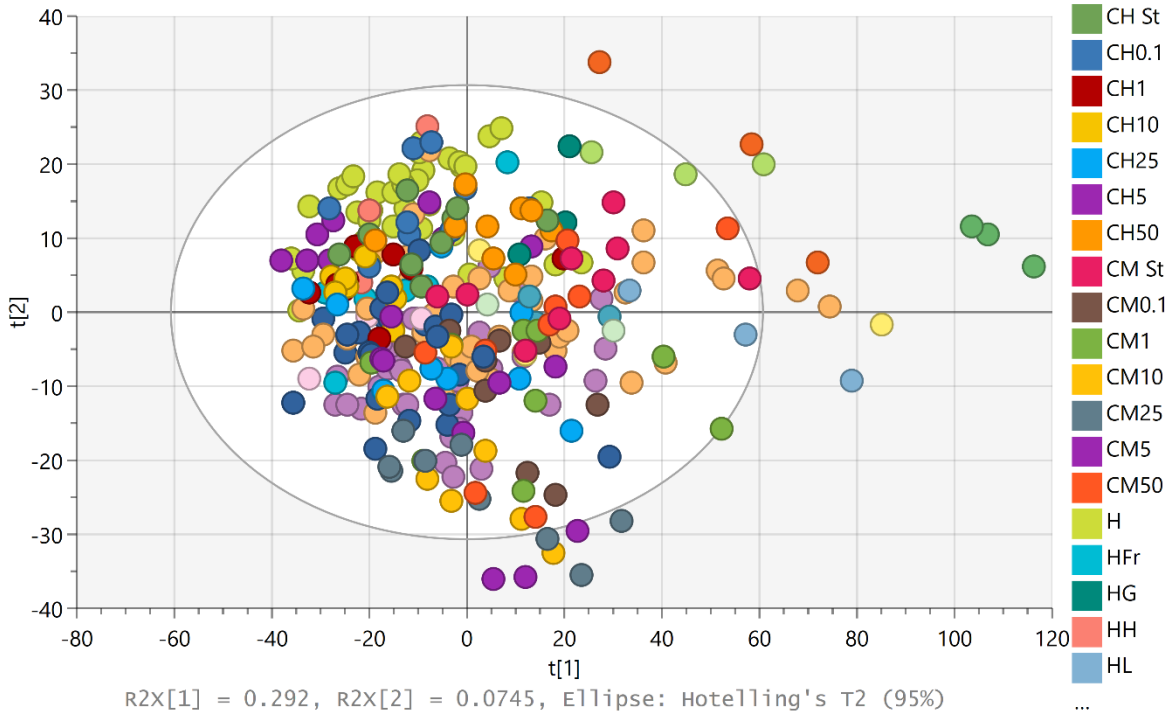
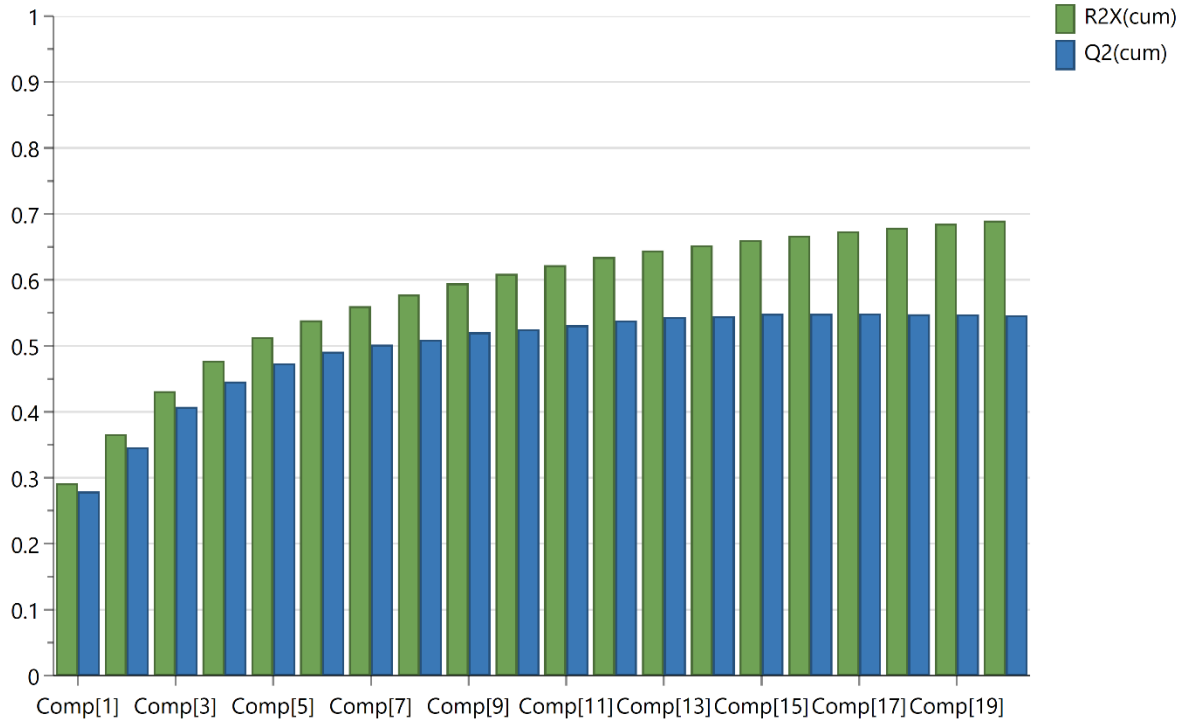
Unsupervised overview of the data





PCA analysis provides an overview of the variance within the data, and any major trends that are clearly ‘visible’. Because of the large number of different groups it is hard to get a clear picture, though usually PC1 (29 % of variation) usually explains analytical variation. Notably ‘MG’ is clearly separated from the rest of the samples

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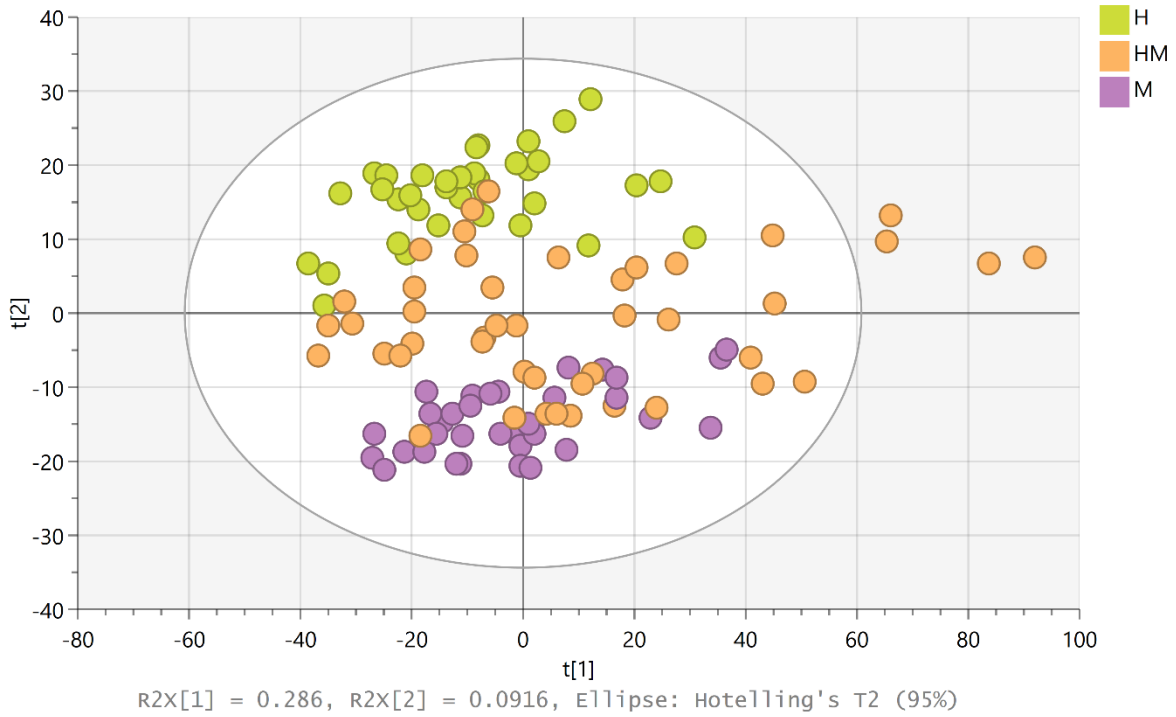
Hoki vs Mackerel vs ‘HM’: PCA



PC2 explains most of the variance between Hoki and Mackerel.
HM appears to be mostly in between the two, but with substantially greater variance

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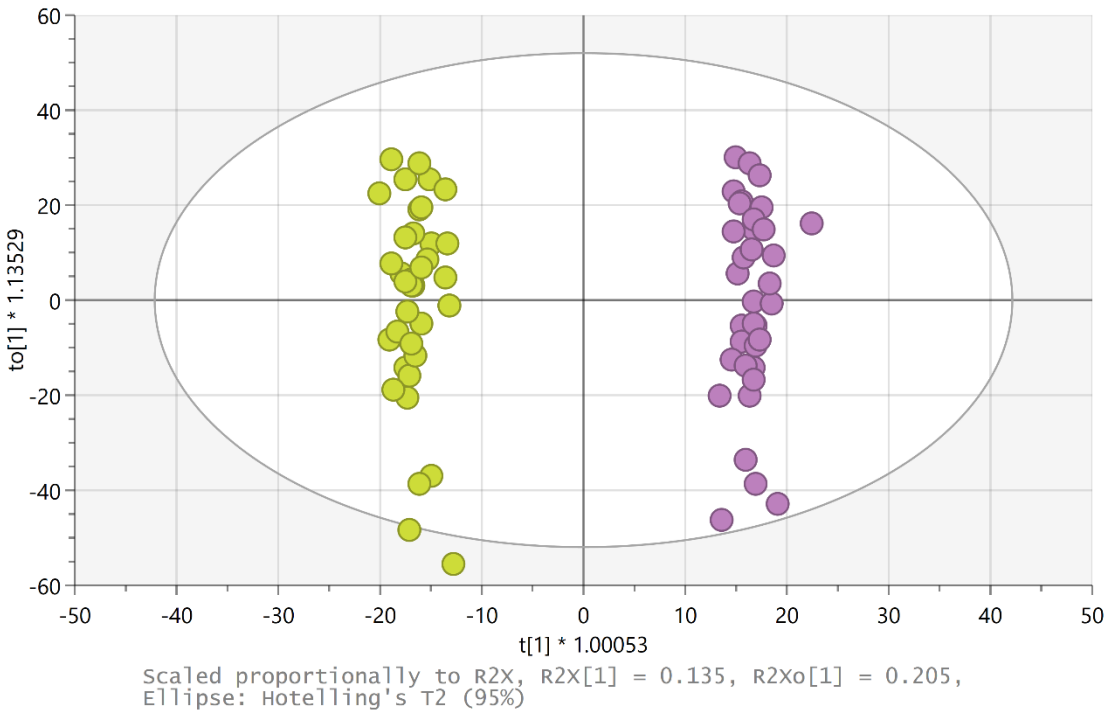
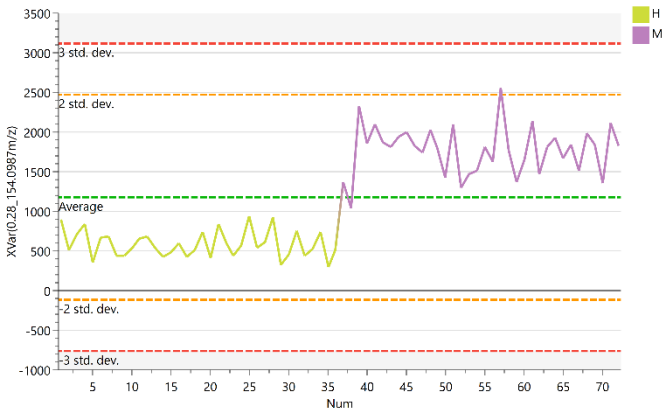
Hoki vs Mackerel: OPLS-DA

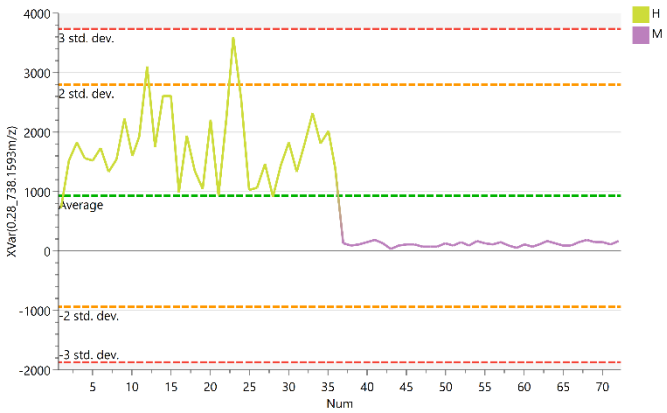


OPLS-DA is a form of supervised analysis (the model is told what groups to try to separate), similar to PLS-DA, except for forcing the difference between groups to be along the first component. The model robustness (Q2) is 0.9, which is excellent.

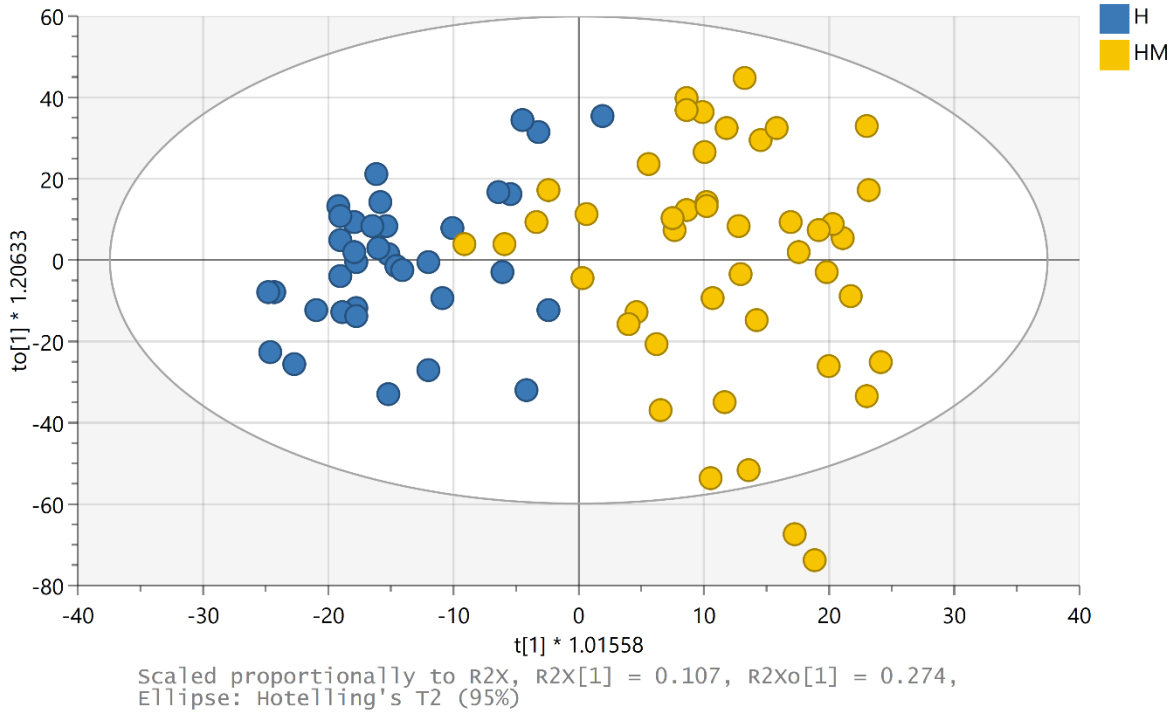
OPLS-DA works best in a two group comparison situation

Examples of 'features' that differ between hoki and mackerel:



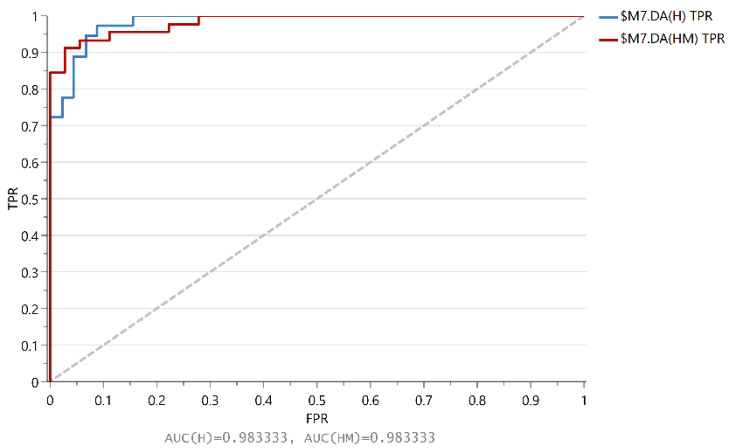


Hoki vs HM: OPLS-DA



In this case, the separation is not as clear cut – the higher variance in the HM data has an impact. The robustness statistic Q2 is 0.37, which is okay. As we have included all 2080 features detected, the next step is to use feature reduction to see if this improves the model.

Removing features with VIP <1 improves the Q2 score to 0.48, with AUC for the ROC is 0.98



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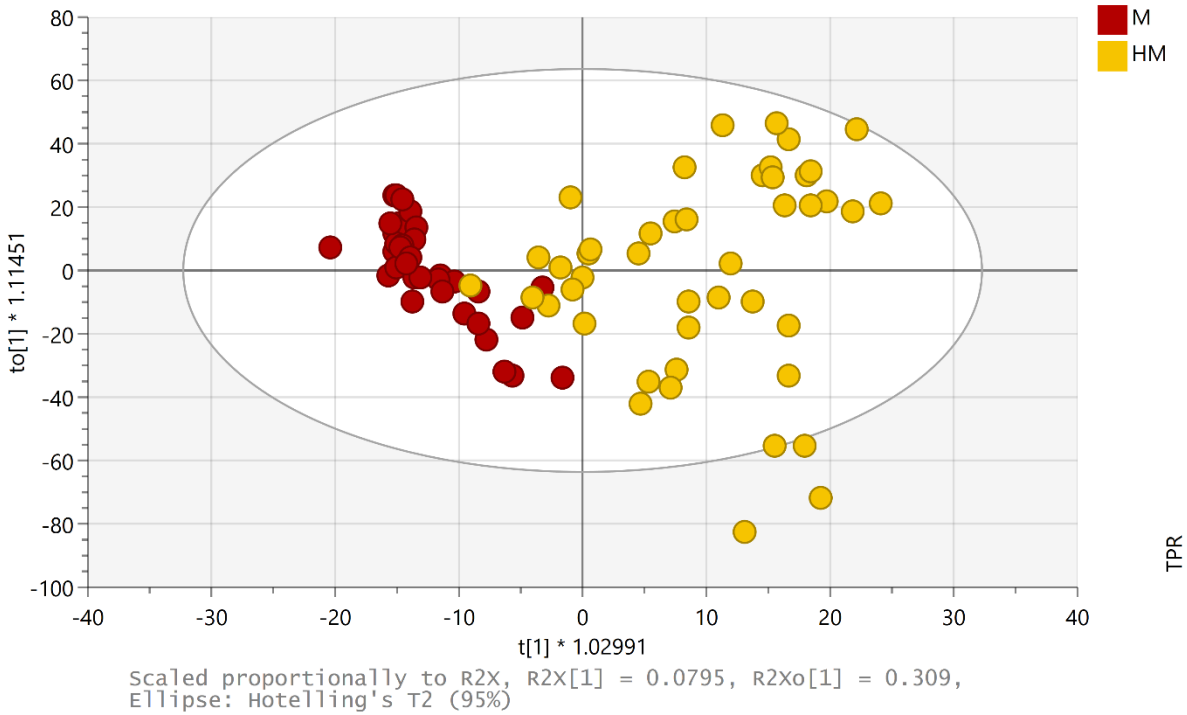
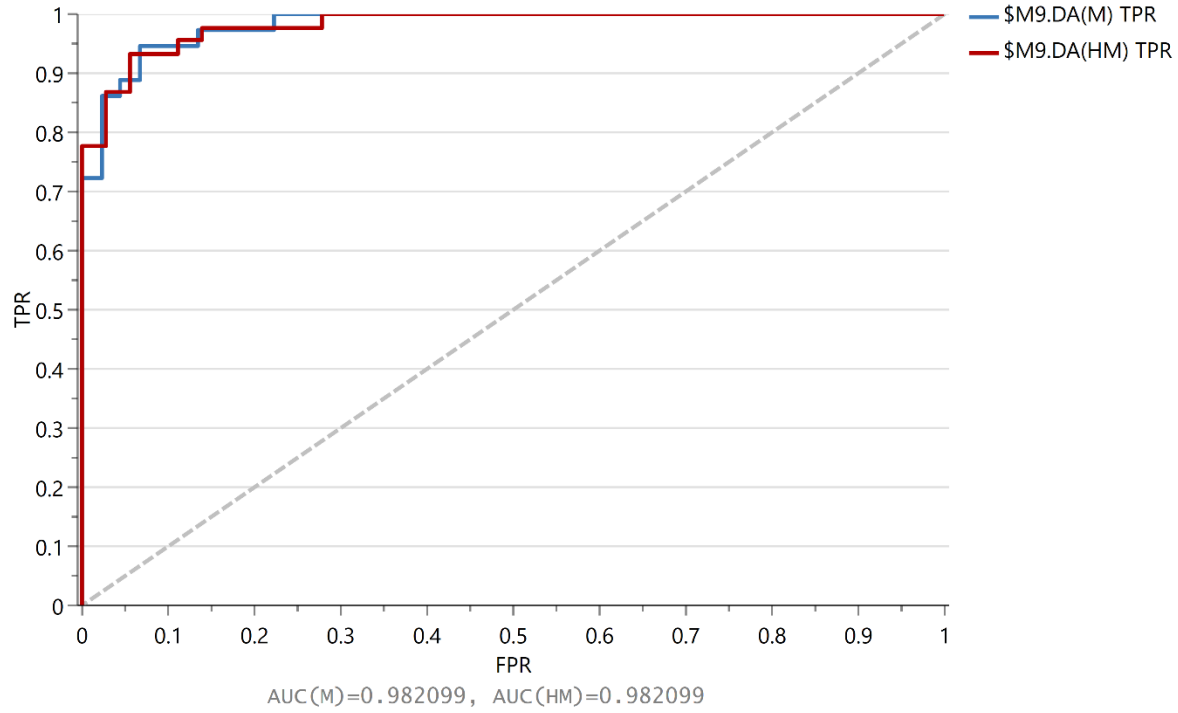
Mackerel vs HM: OPLS-DA



Very similar for Hoki vs HM – the high variance in HM creates some issues. Q2 = 0.36. For the VIP<1 removed model, Q2 increases to 0.46



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Summary

- REIMS was able to distinguish between hoki and mackerel with a very strong model
- Difference between mixed hoki and mackerel was clear but not as strong
- Samples were present in sequential order in the plates, but the plate order randomised – this should avoid any major impact of instrumental drift
- More steps to correct the data for instrumental drift (e.g. QC-based normalisation) should reduce non-species related variation