Interpreting the Results of Peptide Analysis

Custom synthesised peptides from Mimotopes are manufactured under strict high level quality control processes. Yet, because each custom peptide is a new compound, it may present unforeseen problems for us in synthesis, purification or characterization (analysis). You may not be familiar with peptide analysis techniques, so we have prepared this note to help you with interpretation of the results of analysis of your peptide(s).

No single analysis technique reveals all the desired information about a peptide. We use the efficient method of mass spectrometry (MS) for all single custom peptides, and for purified peptides we also analyze by reverse phase HPLC. If you require a wider range of analyses on your peptides than supplied with our standard products, please inquire at the time of placing your next order. Alternatively, we are happy to perform other analyses on your current peptide(s) (at additional cost). Please contact Mimotopes Customer Service for details.

1. Electrospray Mass Spectrometry (ESMS)

ESMS, also known as ion spray mass spectrometry, unlike older methods such as HPLC, amino acid analysis, sequencing etc., gives a variety of both qualitative and quantitative information. Qualitatively, ESMS allows confirmation of the presence of a species with the molecular mass of the correct target peptide, and also allows identification of many of the impurities. In addition to qualitative data, the recent advances in mass spectrometry technology embodied in ESMS also allow quantitation of the charged components of a mixture.

Topics in this article:

We have chosen to apply ESMS to the routine analysis of all our peptides. Spectra are collected as positive ions, obtained by vaporizing the peptide from acidic (trifluoracetic or acetic acid) solution.

The interpretation of ESMS data, as used in quality checking of our peptides, can be summarized:

i. Presence of major peaks corresponding to the known mass-to-charge ratios of the target peptide are taken as confirmation of the presence of the target peptide.

ii. The ESMS Figure supplied is derived from the spectrum of raw mass-to-charge ratio versus ion count. The Figure shows the molecular mass of the species that gave rise to the peaks in the raw spectrum. The data have been simplified by summing the peaks arising from natural isotope distributions for each component, and are plotted at the molecular mass value where the signal is maximum.

The Y-value plotted is the percentage of the total ion count signal contributed by that species. Any peaks present which do not correspond to the monomeric, salt-free target peptide are assigned on the basis of known or possible molecular variants of the target peptide.

Most of these are assignable as impurities (deletions, truncations etc.) but some are identifiable as non-covalent adducts of the target peptide (salts, dimers etc.; see below*). When we specify the purity of the peptide, the ion count contributions of these adduct peaks are included in the total for the target peptide.

iii. The ESMS purity value is the ion count attributable to the target peptide, expressed as a percentage of the total ion count.

Studies in our laboratories [1] have validated quantitative ESMS for estimating the purity of synthetic peptides. The purity as measured by MS and by the commonly used quantitative HPLC method do not always agree. We have chosen to use ESMS for characterization and purity estimation for the "Unpurified" custom peptides, while for the ">70%" and higher-purity custom peptides, the purity is calculated from the area under the analytical HPLC trace (see below) in the conventional way and ESMS is used only for confirmation of the identity of the peptide. This is because quantitative HPLC is presently the most widely-accepted method for this purpose, despite its limitations.

2. Reverse Phase HPLC (RP-HPLC)

This technique gives high resolution of peptides having small differences in structure and is thus good for detecting the presence of impurities with properties significantly different from the target peptide. Quantitation is by measurement of areas under peaks in the absorbance trace (214nm) of the analytical HPLC column effluent. This wavelength predominantly measures peptide bonds. Purity is expressed as the area percent contributed by the main peptide peak, as a proportion of the total area under the curve within the region of the HPLC trace where peptides are known to elute. The main peptide peak is confirmed to be the target peptide using the quantitative information from the ESMS (see above). In any cases of doubt, additional analyses are performed (see below). Please note that the calculations do not include the injection or solvent front peaks present in most HPLC traces.

3. Other Analyses

We offer combined HLPC and MS (known as LC/MS); standard amino acid analysis; sequencing; and chromatography other than RP-HPLC if required. Please inquire for prices and availability.

*Adducts which are commonly seen include the trifluoroacetate salt (114 Dalton additional mass), the ammonium salt (17 Dalton additional mass), and non-covalent polymers (dimers, trimers etc.) of the correct target peptide. Polymeric forms are usually above the molecular mass range shown on the Figure. The percentage of total ion count attributed to species with molecular mass outside the plotted range is printed below the X-axis on the ESMS Figure.

References

[1] Smart, S.S., Mason, T.J., Bennell, P.S., Maeji, N.J. and Geysen, H.M. (1996) High Throughput Purity Estimation and Characterisation of Synthetic Peptides by Electrospray Mass Spectrometry. Int. J. Pept. Protein Res. 47; 47-55.

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