Limitation of sequencing of peptides via CIF (collision induced fragmentation)

Sequencing of peptides is performed by MS^n after CIF. This method has advantages and but also limitations in comparison of Edman degradation.

Sequencing via MS^n

It depends on the length and the structure of the peptide if complete verification of the sequence is possible.

- Usually complete verification is possible for peptides with a length of <20 amino acids.
- Sequencing of peptides with a ring structure as eg. Octreotide is only possible after reduction of the disulfide bridge.
- For longer peptides amino acid sequencing at the termini might be not achieved.
- It is possible that fragmentation of an amino acid bonds in the middle of the peptide can be not achieved.
- If a peptide contains a couple of basic amino acids as Lys, Arg and His electrospray ionization results in a high loaded molecule so that the concentration of 3- or 2-fold loaded molecules is very low even when the concentration of formic acid is reduced. In this case reliable annotation might fail and in addition the fragments are still high loaded.

Differences to Edman degradiation:

The main differences are of course a result of the total different technique. Whereas Edman degrade successively one amino acid after the other, the CIF approach degrade the peptide in all possible fragments that are detected by mass spectroscopy. Therefore

- CIF sequencing is not useful for de-novo sequencing due to the complexity of the possible mass pattern.
- Edman sequencing starts at one terminus. If during Edman degradation one amino acid is not to cleave Edman degradation fails from this point whereas sequencing via CIF continues after a gap.