CLAIMS

1. A method for determining the amino acid sequence and mass of a polypeptide or mixture of polypeptides, comprising the steps of:
   a) preparing polypeptide sample by dissolving the polypeptide in HCl and adjusting pH to between 7.5 and 8.5 by Tris;
   b) digesting the polypeptide sample by addition of endoproteinase Glu C in ratio of 25:1;
   c) reducing the digested sample of step (b) by addition of 1M dithiothreitol;
   d) performing HPLC analysis followed by mass spectrometry analysis of the polypeptide of step (c);
   e) comparing the molecular mass of the polypeptide with the molecular mass of a corresponding known polypeptide, thereby determining the identity of the polypeptide;

   wherein organic solvent used in step (d) is a non-salt based buffer and the method allows sequence confirmation of multiple fragments at the same time.

2. The method as claimed in claim 1, wherein the non-salt based buffer is selected from a group consisting of acetonitrile, formic acid, TFA, or a combination thereof.

3. The method as claimed in claim 1, wherein the time required for steps (d) and (e) is about 40 minutes.

4. The method as claimed in any preceding claims, wherein the polypeptide(s) is insulin or insulin analogs.

5. The method as claimed in claim 4, wherein the polypeptide is selected from a group consisting of aspart, lispro and glargine.

6. The method as claimed in claim 1, wherein the polypeptides can differ at least by one amino acid.

7. The method as claimed in any preceding claims, wherein analyzed fragments of polypeptide(s) of step (e) have a mass ranging from 0.4kDa to 8kDa.