Orbitrap MS Analysis of Insulins

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I. BACKGROUND

Identification and characterization of three analogues of Insulin via MS, Tandem Mass spectrometry (MS^2) and Collision cross section (CCS) using Orbitrap MS. (*Analytical Chemistry* 2019).

II. METHODS AND MATERIALS

A. MATERIALS

1) Instruments:

- Agilent HPLC (1260 Infinity)
- RP Column: Agilent Zorbax SB-C8; 4,6 x 50 mm; 1,8 μm
- Thermo Scientific LTQ Orbitrap XL

2) Chemicals:

- mobile phase A: 0,1% TFA
- mobile phase B: IPA/ACN/H_2O (7: 2: 1) with 0,1% TFA

B. METHODS

As displayed in Table I insulin human-Actrapid and insulin lispro-Humalog have the identical sum formula and therefore the same molecular weight (5806,66 Da). There is however a structural difference in the α and β chain of these molecules which allows identification due to differences in masses of the fragments. While the α chain of human insulin contains Asn-Pro, lispro has a Asn-Lys. On the β chain, lispro has Lys-Thr (monoisotopic mass 0 247,16) and human insulin has Pro-Thr (monoisotopic mass = 216,12). In order to achieve the desired fragments tandem-mass spectrometry has to be used in order to identify lispro and human insuline.

Insulin	Sum Formula	Molecular
Insulin human - Actrapid	C257 H383 N65 O77 S6	5806,66
Insulin glargin - Lantus	C267 H404 N72 O78 S6	6062,85
Insulin lispro - Humalog	C257 H383 N65 O77 S6	5806,66

TABLE I: The analysed insulin samples with their sum formula and molecular weight

III. RESULTS

The identification of lispro and human insuline is demonstrated in figure 1, where the expected fragments (m(lys-Thre (-H_2O = 247,31 Da, m(Pro-Thre (-H_2O = 216,24Da) are identified in the respective mass spectra (Fig. 1a, Fig. 1c). Figures 1b and 1c provide a zoomed in view with information for control of the monoisotopic mass (see. METHODS) via Equation 1.

$$M = n(\frac{m}{z}H^+) \quad (1)$$

Molecule	m/z	Ccs	Z
Human	968.2773	1985.2	6
Lispro	968.2765	1984.2	6
Glargin	1010.8099	1926.8	6
Raffinose	527.1475	208.6	1
Maltotriose	527.1595	212.4	1

TABLE II

The identification of Sample 1 (human) and Sample 2 (lispro) leads to their proper labeling in Figure 3, even thought, masses calculated by Equation 1 lead to no appropriate identification. A demonstration of flow injection is displayed in figure 2, where it is clearly visible that more peaks are present with this method. An advantage of this method is the faster and cheaper analysis. Another methode for seperation is Ion-mobility. It is used to identify ionized molecules in gas phase based on their mobility in a carrier buffer gas. In our experiment it was used to determine the components in sample 1 (human insulin), sample 2 (insulin lispro), sample 3 (insulin glargine), raffinose and maltotriose. IMS can detect the m/z ratio As averaged collision cross section (CCS), values can obtain from databasis . This approach was used in our experiment, determination can be calculated when the 3D structure of the molecule is known (Tab. II)

IV. DISCUSSION

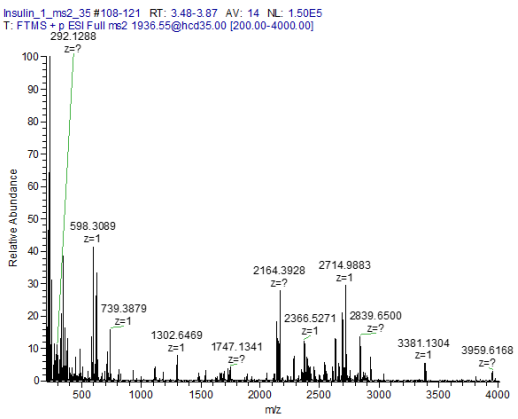
Several techniques for resolving structures with equal mass are present in this paper. The methods chosen for seperation and identification worked well and are suitable for experiments regarding insuline identification.

A. Experimental environment

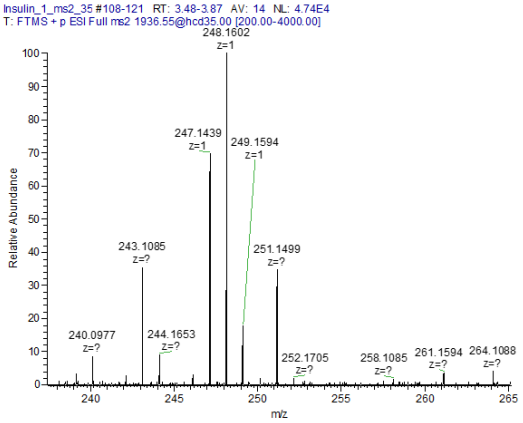
The experiment was performed at TNF Turm JKU, Linz, Austria on 27.06.2019 under the supervision of Markus Himmelsbach.

REFERENCES

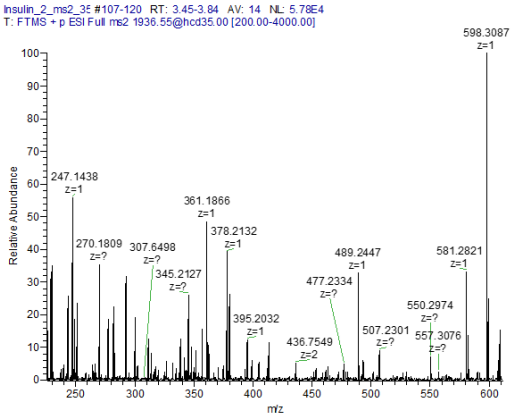
[19] *Analytical Chemistry*. SOPs Lab Course in Instrumental Analytical Chemistry for Molecular Biology. 2019.



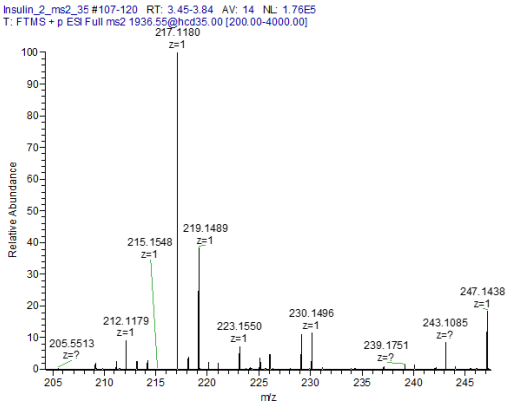
(a) Mass spectrum identified as human insulin by Figure 1b.



(b) Inserting the variables n and $\frac{m}{z}$ into Equation 1 results in 247,16 Da. This identifies the sample as human insuline.

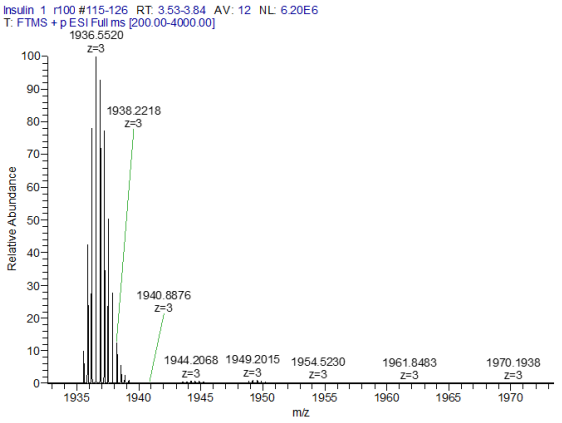


(c) Mass spectrum identified as human insulin by Figure 1d.

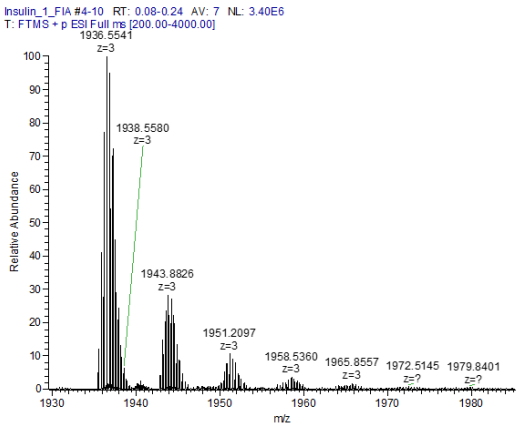


(d) Inserting the variables n and $\frac{m}{z}$ into Equation 1 results in 216,12Da. This identifies the sample as lispro insuline.

Fig. 1: Identification of human and lispro insuline using MS^2.

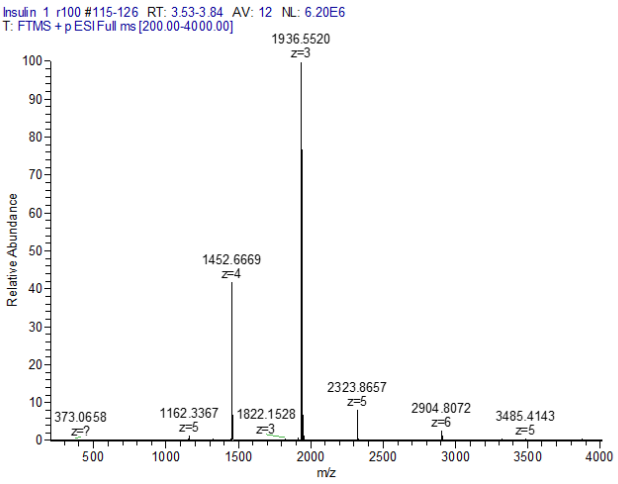


(a) Spectrum with chromatographical seperation.

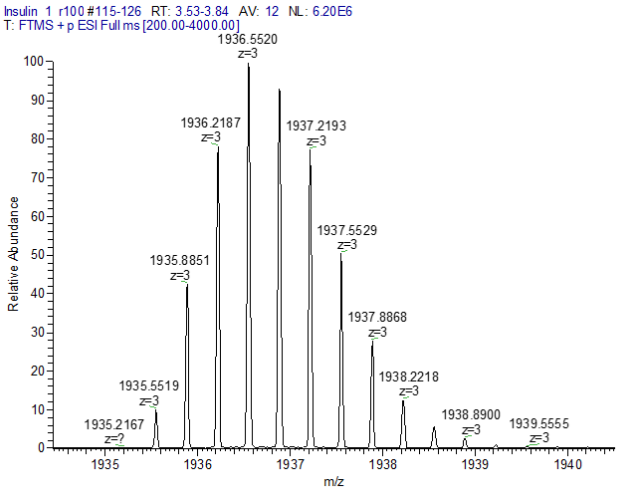


(b) Flow injection without seperation.

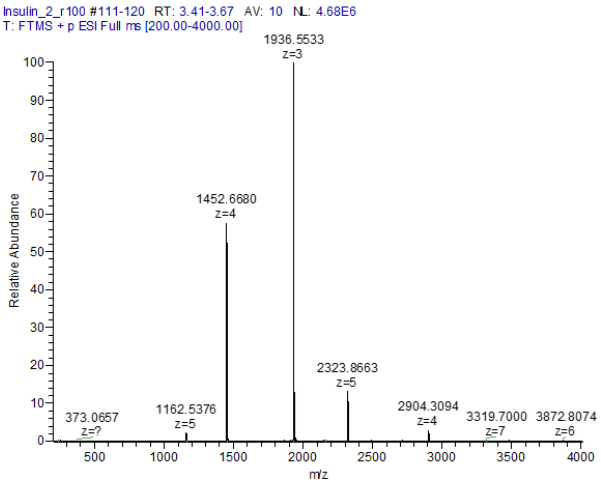
Fig. 2: Comparison of flow injection and separated injection.



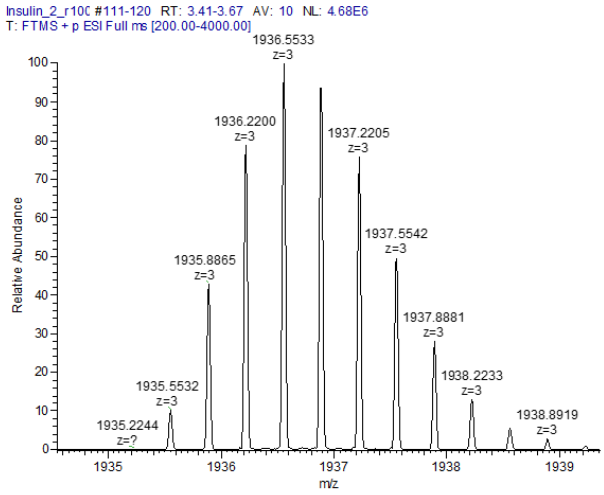
(a) Mass spectrum identified as human insulin by Figure 1b.



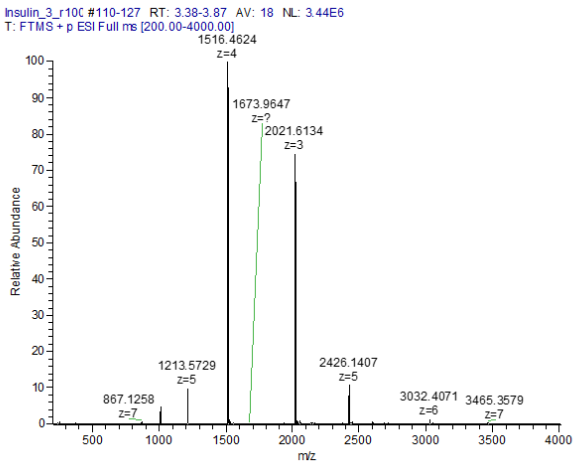
(b) Inserting the variables n and $\frac{m}{z}$ into Equation 1 results in 5 806. 66 Da.



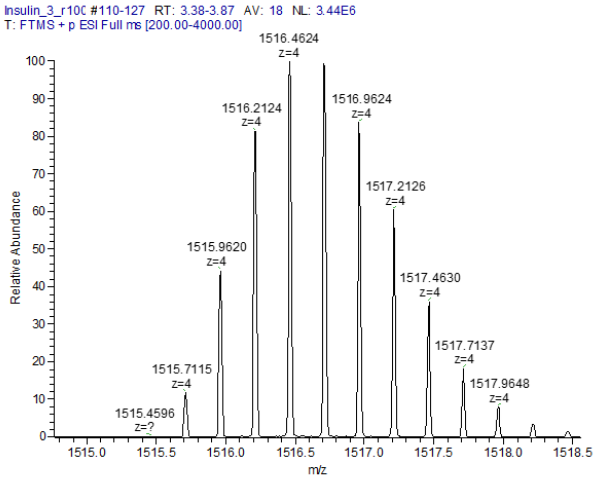
(c) Mass spectrum identified as lispro insulin by Figure 1b.



(d) Inserting the variables n and $\frac{m}{z}$ into Equation 1 results in 5 806. 66 Da.



(e) Mass spectrum identified as glargin insulin by Figure 3f.



(f) Inserting the variables n and $\frac{m}{z}$ into Equation 1 results in 6061, 85 Da. This identifies the sample as glargin insuline.

Fig. 3: Three insuline samples mass spectrums