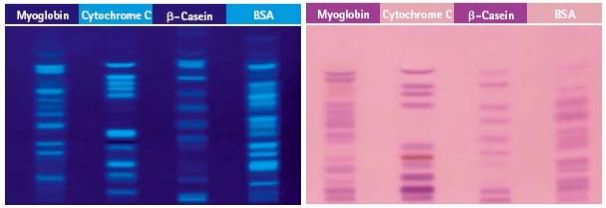
Controlling the Insulin purification process using HPTLC - MS

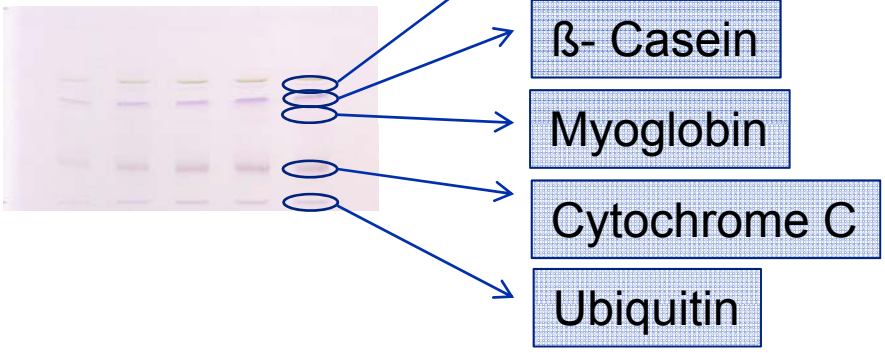
Michael Schulz MM LER CP
08.07.2011

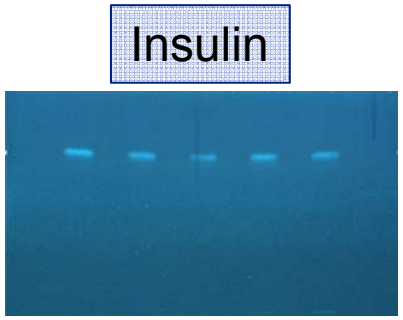
HPTLC - MS of peptides from tryptic digest of proteins



[1] Pasilis, S. P. *et al.* Anal. Bioanal. Chem. 391 (2008) 317
[2] Pasilis, S. P. *et al.* J. Mass Spectrom. 43 (2008) 1627
[3] Emory, J. F. *et al* Eur. J. Mass Spectrom. 16 (2010) 21
[4] Schulz, M. *et al* CBS 106

HPTLC – MS of proteins





Insulin human

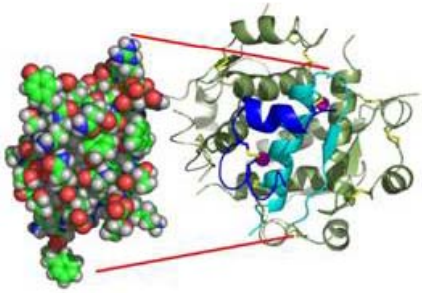
A-Chain: GIVEQCCTSICSLYQLENYCN

B-Chain: FVNQHLCGSHLVEALYLVCGERGFFYTPKT

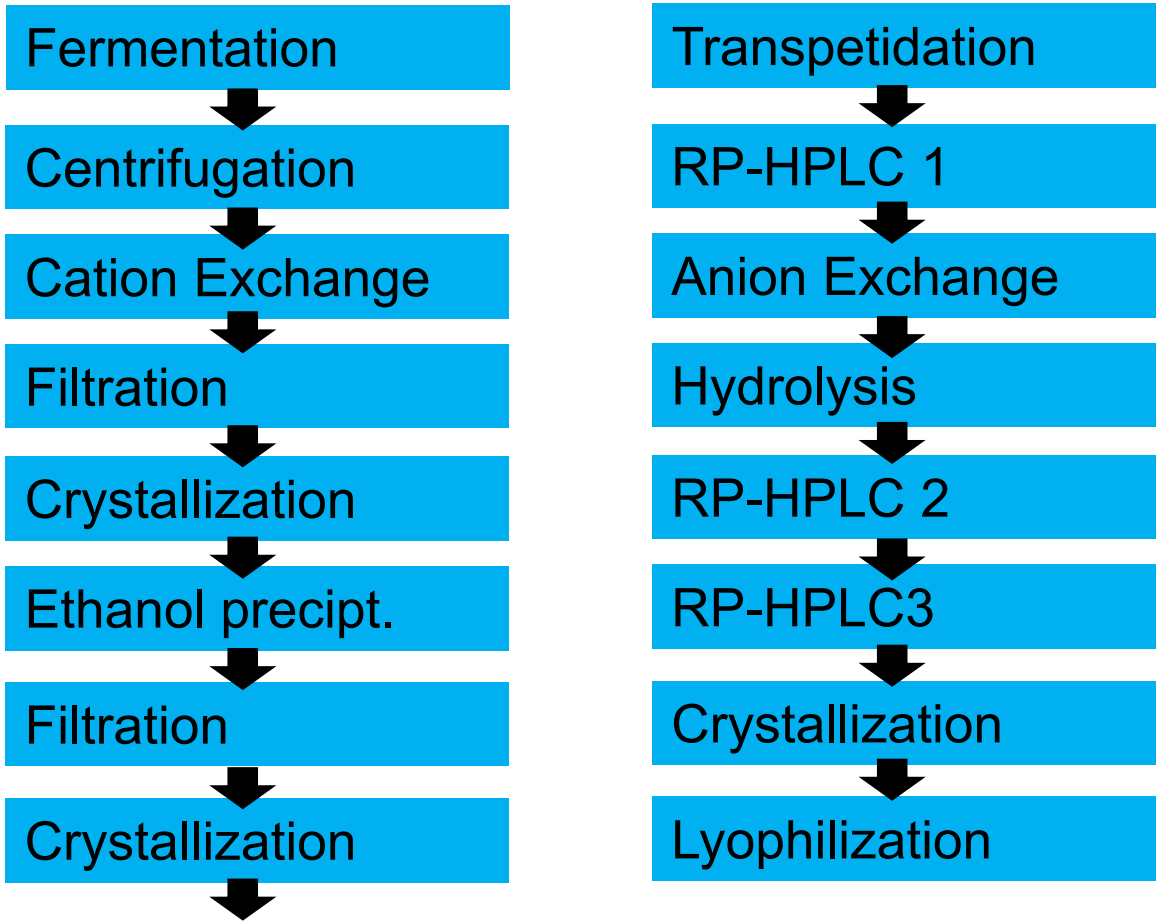
→ 51 Amino acids

→ $C_{257}H_{383}N_{65}O_{77}S_6$

→ 5807 Da



Insulin purification process



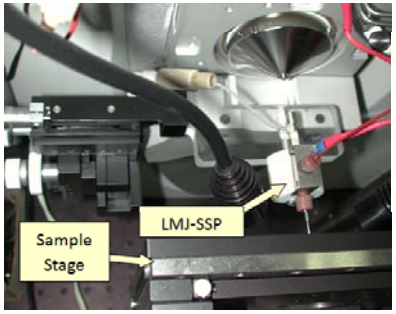
Mollerup at all, Encyclopedia of Industrial Biotechnologie

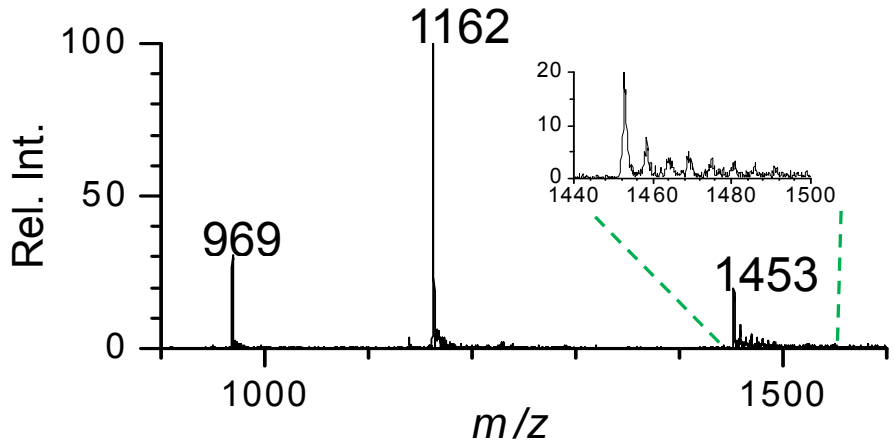
Analysis of human Insulin after temperature treatment with HPTLC - LMJ - SSP - DESI - MS

Analysis of human Insulin after temperature treatment with HPTLC - LMJ - SSP - DESI - MS

MS performed by Organic and Biological Mass Spectrometry Group, Chemical Science Division, Oak Ridge National Laboratory, Gary van Berkel

- full scan modus possible
- hydrophobic impregnation of Silica or Cellulose plates

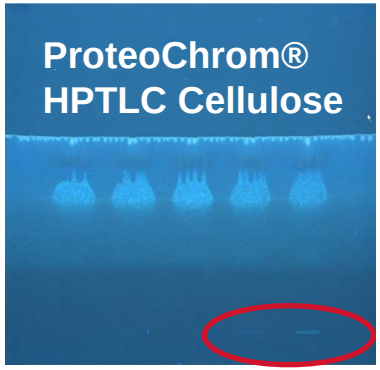


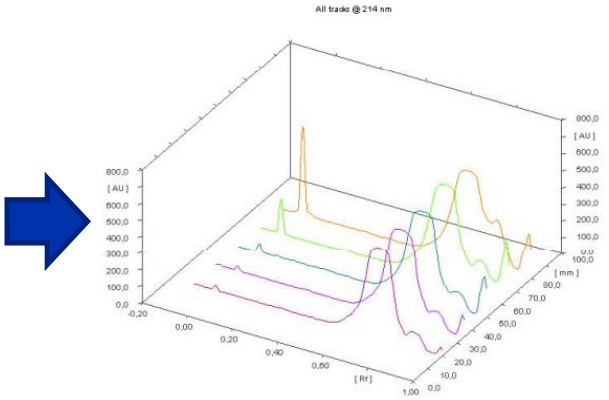


Analysis of human Insulin after temperature treatment with HPTLC - LMJ - SSP - DESI - MS

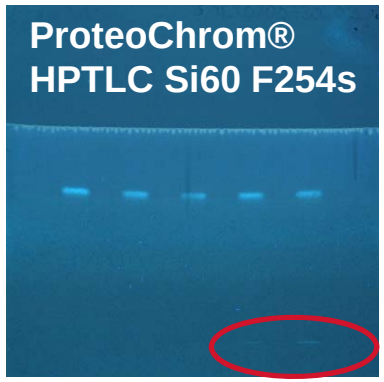
Temperature treatment:
(1) 0 h at 50°C
(2) 24 h at 50°C
(3) 48 h at 50°C
(4) 72 h at 50°C
(5) 96 h at 50°C

C. Yomota et al., J Chromatogr A 721 (1996) 89 "... A-21DHI was prepared by placing HI in 0,01 M HCl at 40°C for 48 h..."



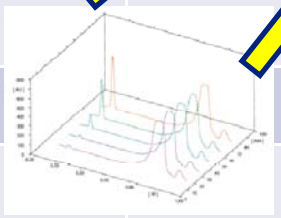


1 2 3 4 5



Analysis of human Insulin after temperature treatment with HPTLC - LMJ - SSP - DESI - MS

Time at 50°C	Peak Area at spotting position	Peak Area Insulin
0	153	18168
24	156	17513
48	719	17077
72	2146	15811
96	3033	15446(85%)



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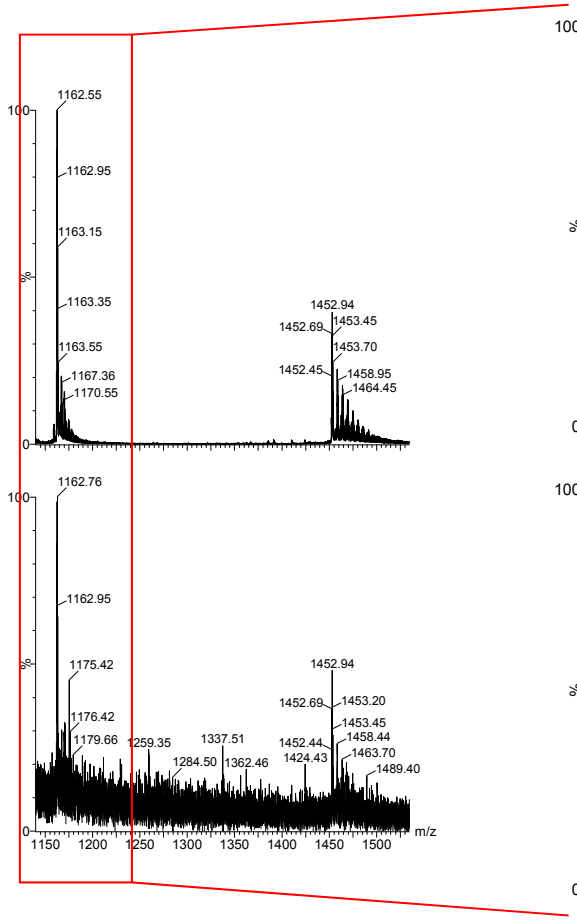
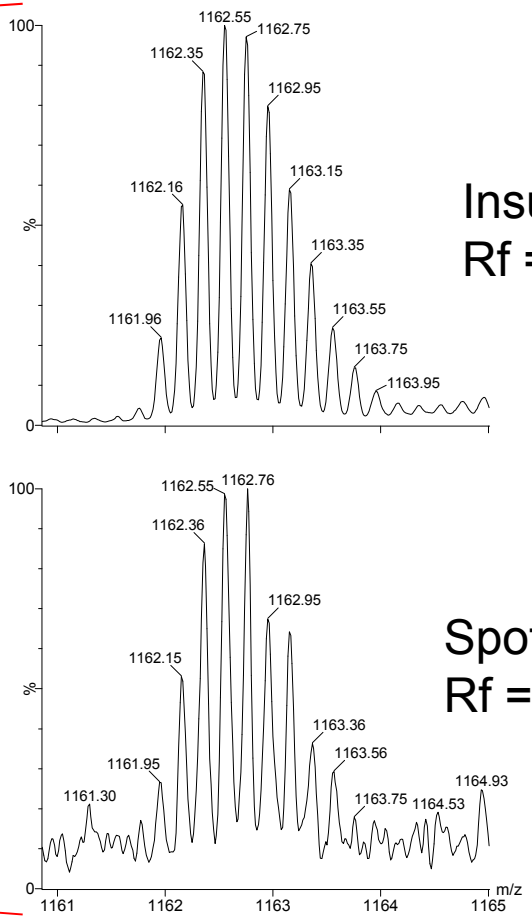




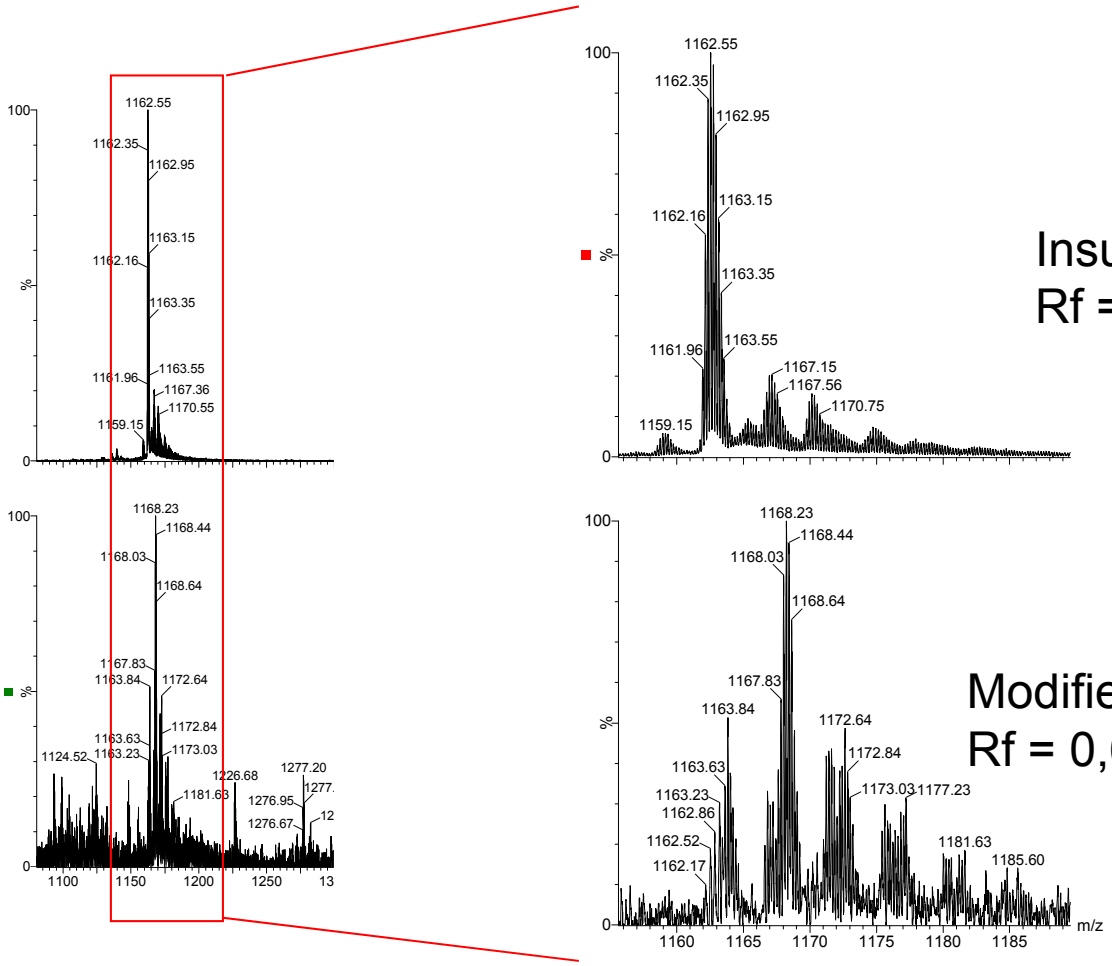
Insulin
Rf = 0,8

5808 Da

Spotting point
Rf = 0

5808 Da

Analysis of human Insulin after temperature treatment with HPTLC - LMJ - SSP - DESI - MS



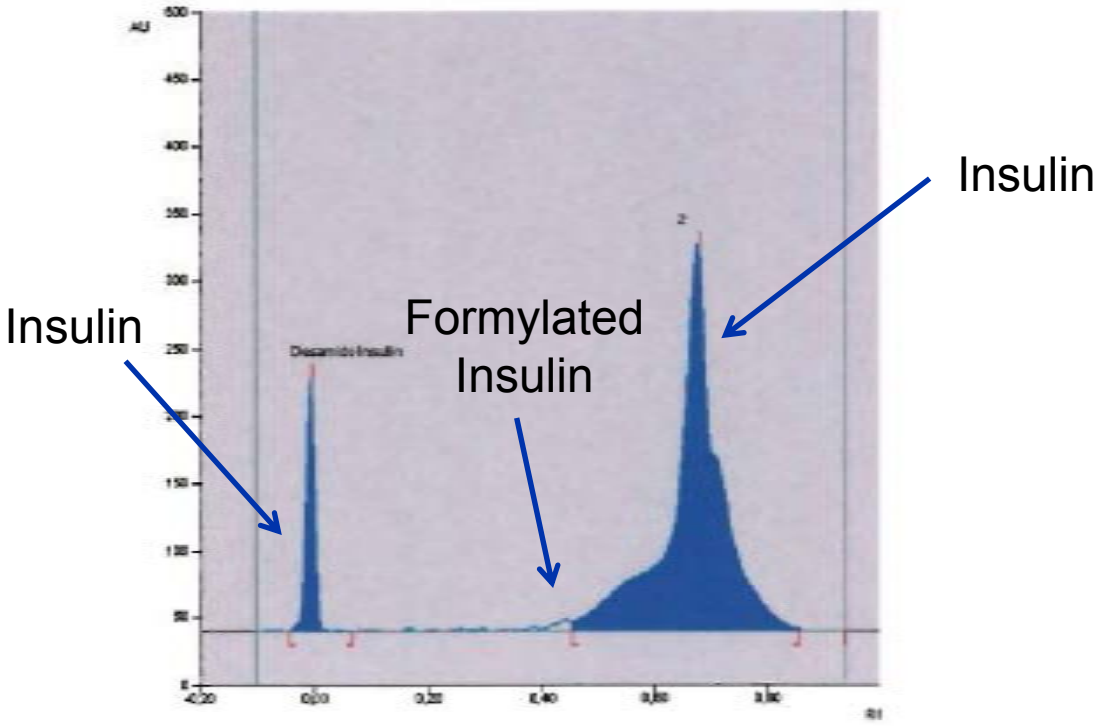
Insulin
Rf = 0,8

5808 Da

Modified insulin
Rf = 0,6

5836 Da

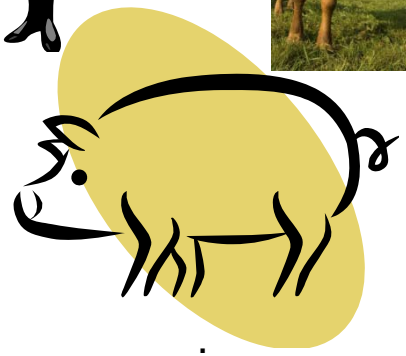
Analysis of human Insulin after temperature treatment with HPTLC - LMJ - SSP - DESI - MS



Identification of Insulin from different species using the TLC MS interface

human

bovine



porcine

Identification of Insulin form different species using the TLC MS interface

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→ 51 Amino acids

→ $C_{257}H_{383}N_{65}O_{77}S_6$

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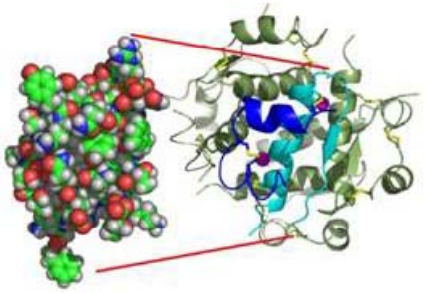


Insulin human

Identification of Insulin form different species using the TLC MS interface

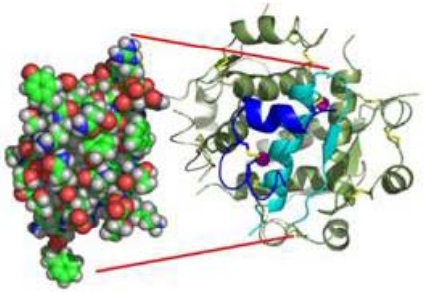
A-Chain: GIVEQCCTSICSLYQLENYCN

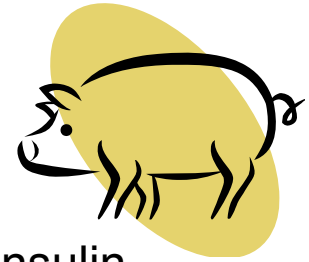
B-Chain: FVNQHLCGSHLVEALYLVCGERGFFYTPK**A**

→ 51 Amino acids

→ $C_{256}H_{381}N_{65}O_{76}S_6$

→ 5778 Da





Insulin porcine

Identification of Insulin form different species using the TLC MS interface

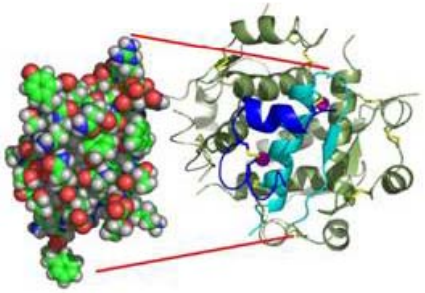
A-Chain: GIVEQCC**A**S**V**CSLYQLENYCN

B-Chain: FVNQHLCGSHLVEALYLVCGERGFFYTPK**A**

→ 51 Amino acids

→ $C_{254}H_{377}N_{65}O_{76}S_6$

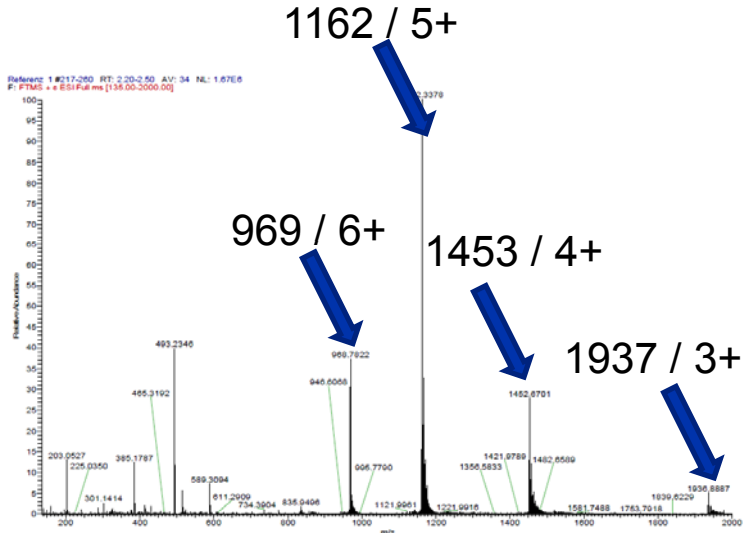
→ 5733 Da



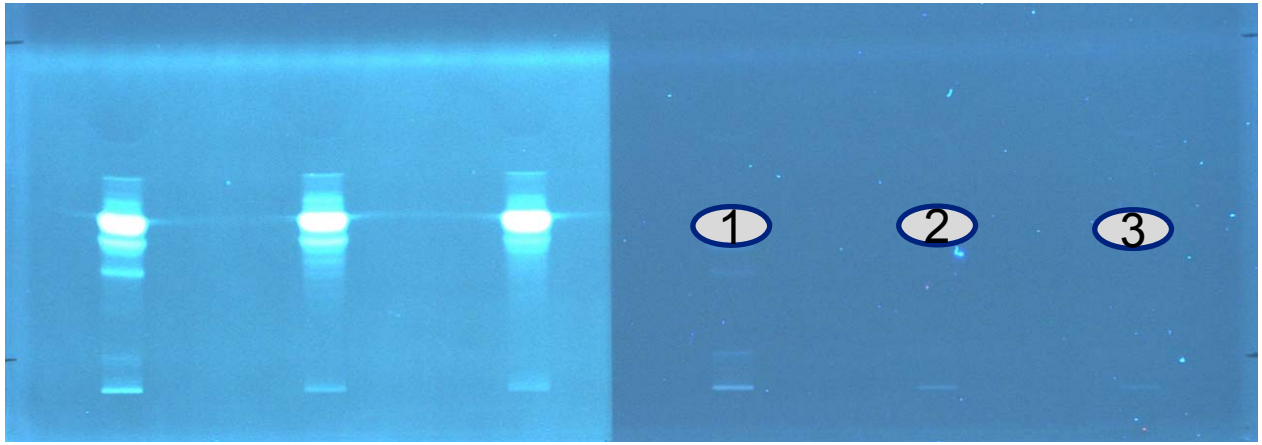
Insulin bovine

Identification of Insulin form different species using the TLC MS interface

Insulin human identified with the TLC MS Interface coupled with ESI -MS (Thermo LTQ XL Orbitrap)

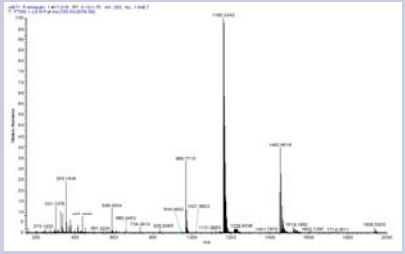
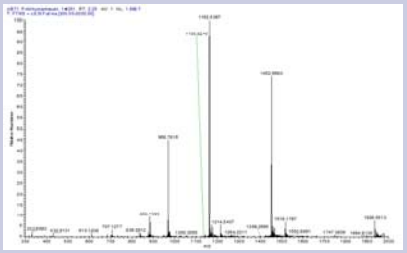
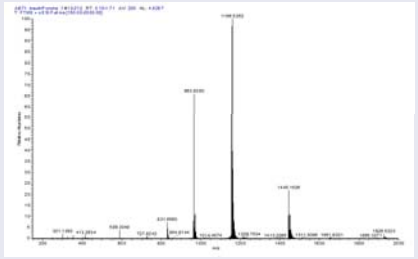
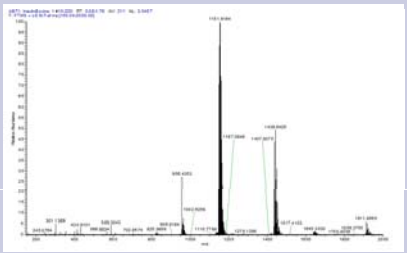
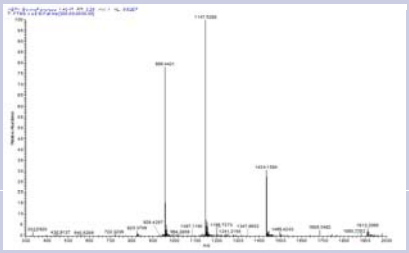


Identification of Insulin form different species using the TLC MS interface



Chromatographic conditions	
Stationary Phase	ProteoChrom® HPTLC Silica gel 60 F254s
Mobile Phase	2-butanol / pyridine / NH3 / water (39 / 34 / 10 / 26 [mL])
Migration distance	5 cm
Migration time	50 min

Identification of Insulin form different species using the TLC MS interface

	Reference spectrum	From HPTLC Plate
Insulin human (1)		
Insulin porcine (2)		
Insulin bovine (3)		

Identification of Insulin form different species using the TLC MS interface

Insulin human	Insulin porcine	Insulin bovine
1935,55 [M+3H] 3+ Mass accuracy 0,2 ppm	**1925,55** [M+3H] 3+ Mass accuracy 0,4 ppm	**1910,87** [M+3H] 3+ Mass accuracy 0,4 ppm
1451,92 [M+4H]4+ Mass accuracy 0,4 ppm	**1444,41** [M+4H]4+ Mass accuracy 0,2 ppm	**1433,41** [M+4H]4+ Mass accuracy 0,3 ppm
1161,73 [M+5H]5+ Mass accuracy 1,1 ppm	**1155,73** [M+5H]5+ Mass accuracy 1,2 ppm	**1146,93** [M+5H]5+ Mass accuracy 1,3 ppm
968,28 [M+6H]6+ Mass accuracy 0,7 ppm	**963,28** [M+6H]6+ Mass accuracy 1,1 ppm	**955,94** [M+6H]6+ Mass accuracy 0,7 ppm
5804 Dalton	**5774 Dalton**	**5730 Dalton**

Summary

- HTLC - MS of Insulin with different techniques and mass spectrometers successful
- Protein structure intact after TLC development. Expected molecular masses found
- Small differences in molecular mass can be detected with high accuracy
- Selectivity for insulin impurities shown