DruQuaR

UNIVERSITEIT GENT

FACULTY OF PHARMACEUTICAL SCIENCES

Peptide profiling of internet obtained Cerebrolysin using HPLC-ESI-IT & UHPLC-Q-IM-TOF mass spectrometry

Bert Gevaert[a], **Matthias D'Hondt**[a], **Nathalie Bracke**[a], **Han Yao**[a], **Evelien Wynendaele**[a], **Johannes Petrus Cornelis Vissers**[b], **Martin De Cecco**[b], **Jan Claereboudt**[b] and **Bart De Spiegeleer**[a]

[a] **Drug Quality and Registration (DruQuaR) group, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium**
[b] **Waters, ConneXion Business Park, Brusselsesteenweg 500, B-1731 Zellik, Belgium**

*Corresponding author: bart.despiegeleer@ugent.be (*O. Ref.*: 2015-208b)

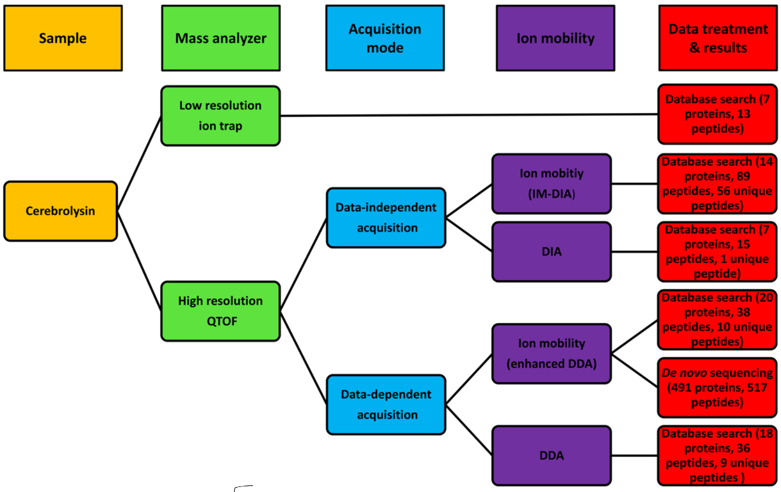
INTRODUCTION

- **Cerebrolysin** is a parenteral **peptide preparation** produced from **porcine brain** proteins and is globally available on the **internet**. Nevertheless, until now, its exact chemical **composition** is **unknown**.
- The chemical composition of a product of this origin is very important for **consistent quality, safety and efficacy**, and seen the various contradictory results reported about its content, this study aims to clarify its peptide composition using and comparing different LC-MS and data-treatment techniques

METHODS

- **HPLC-ESI/IT MS** combined with pig protein database search.
- **UHPLC- ESI/QTOF HDMS** with 2 acquisition modes (data-dependent analysis (DDA) and data-independent analysis (DIA)) both with and without ion mobility separation => 4 acquisitions; combined with database search and *de-novo* sequencing.

RESULTS and DISCUSSION



Sample

Mass analyzer

Acquisition mode

Ion mobility

Data treatment & results

Cerebrolysin

Low resolution ion trap

Database search (7 proteins, 13 peptides)

High resolution QTOF

Data-independent acquisition

Ion mobitiy (IM-DIA)

Database search (14 proteins, 89 peptides, 56 unique peptides)

DIA

Database search (7 proteins, 15 peptides, 1 unique peptide)

Data-dependent acquisition

Ion mobility (enhanced DDA)

Database search (20 proteins, 38 peptides, 10 unique peptides)

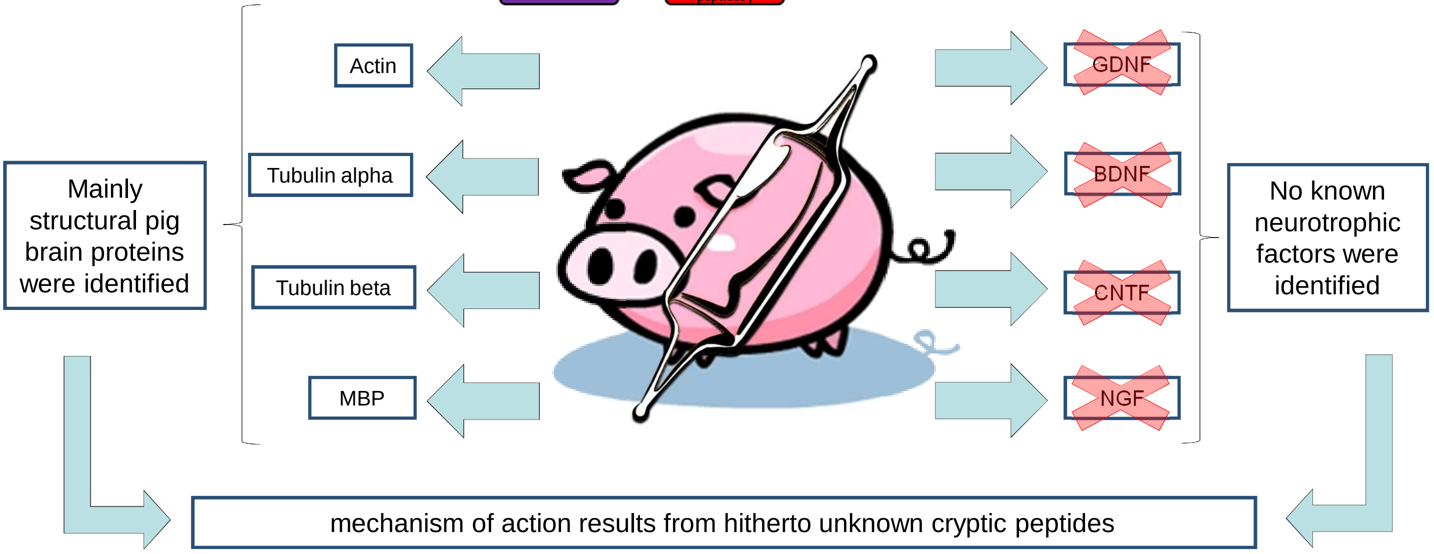
De novo sequencing (491 proteins, 517 peptides)

DDA

Database search (18 proteins, 36 peptides, 9 unique peptides)

UniProt access number	Sequence	%[a]
ACTS_PIG	AVFPSIVGRP	2.6%
ACTS_PIG	TEAPLNPK	9.0%
F1S263_PIG	ELKSLEEEL	3.0%
I3LBD7_PIG	LDKPIQEV	3.7%
I3LJE2_PIG	DHVVPEPGTS	3.7%
K7GLT8_PIG	KIPVGPETLG	2.6%
MBP_PIG	FSWGAEGQKPG	3.1%
MBP_PIG	SWGAEGQKPGFG	9.9%
TBB5_PIG	HSLGGGTGSGMGT	3.7%
TBB5_PIG	LVDLEPGTMDSV	4.2%

[a] Normalized MS/MS total ion current



Actin

Tubulin alpha

Tubulin beta

MBP

Mainly structural pig brain proteins were identified

~~GDNF~~

~~BDNF~~

~~CNTF~~

~~NGF~~

No known neurotrophic factors were identified

mechanism of action results from hitherto unknown cryptic peptides

CONCLUSION

By LC-MS, we identified 638 unique peptides in an internet obtained Cerebrolysin sample which is claimed to have nootropic properties. No fragments of known nootropic proteins are however identified and the reported biological effects are thus likely originating from peptides from other precursors than BDNF, GDNF, NGF and CNTF. The added value of high resolution MS/MS, including ion mobility separation, is clearly demonstrated by the number of identified peptides and unique sequences recovered compared to the other mass analyzers and acquisition techniques.

REFERENCES

Gevaert B, D'Hondt M, Bracke N, Yao H, Wynendaele E, Vissers JP, De Cecco M, Claereboudt J, De Spiegeleer B. Peptide profiling of Internet-obtained Cerebrolysin using high performance liquid chromatography - electrospray ionization ion trap and ultra high performance liquid chromatography - ion mobility - quadrupole time of flight mass spectrometry. Drug Test Anal. 2015 May 28. doi: 10.1002/dta.1817