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Automated Development of a Quantitative Method for Botulinum Neurotoxin E3 using Pinpoint Software and a TSQ-Vantage

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Botulinum neurotoxins(BoNTs) cause the disease botulism, which can be lethal if untreated. Quantification of BoNT can aid in development of effective medical countermeasures. Historically, the mouse bioassay has been used for quantification of BoNT. However, this method is highly inaccurate and requires the use of animals. Therefore, there is a need to develop a rapid quantitative method for BoNTs. We have developed an automated method for quantification of BoNT/E3, based on quantification of peptides generated from a digest of BoNT/E3.

This method involves automated detection of proteins using selective reaction monitoring (SRM). Automation is important to improve the throughput of SRM quantitative method development. This method uses available data-dependent acquisitions acquired on an Orbitrap-Elite.Data wasprocessed in Proteome Discoverer, and imported as a spectral library into Pinpoint. Peptides were *in-silico* digested from the BoNT/E3 sequence using trypsin, and matched to the spectral library information. SRM transitions were selected by Pinpoint.

To test this method, a series of calibrators were prepared in reaction bufferusing a BoNT/E3 standard; calibrators were injected along with a low, medium and high quality control set. The samples were digested with trypsin to generate peptides for detection by SRM on the TSQ-Vantage.We have determined an LOD of 3.9 ng/mL and LLOQ of 8.0ng/mL.Average intraday repeatability was 3%, 10% and 1% for the low, medium and high quality controls. Inter-day reproducibility was 2%, 9% and 3% for the low, medium and high quality controls.

Biography

Jakub Baudys has completed his B.S. at the age of 24 from the University of Utah; he is currently an M.S. graduate level student at the Public Health Practice program at the University of South Florida. He has 13-years of mass spectrometry experience ranging from detection of drugs of abuse and diuretics at the Center for Human Toxicology, to work on bio-analytical assay development for an Antibody Drug Conjugate project at Genentech. He is employed as an analytical chemist at the National Center for Environmental Health (Division of Laboratory Sciences). He has co-authored 14-papers in reputed journals.