

I FEEL SURE THAT THERE ARE MANY PROBLEMS IN CHEMISTRY WHICH COULD

BE SOLVED WITH FAR GREATER EASE BY THIS THAN BY ANY OTHER METHOD.

THE METHOD IS SURPRISINGLY SENSITIVE – MORE SO EVEN THAN THAT OF

SPECTRUM ANALYSIS, REQUIRES AN INFINITESIMAL AMOUNT OF MATERIAL,

AND DOES NOT REQUIRE THIS TO BE SPECIALLY PURIFIED.

de novo hits

■ HCD

■ CID

■ EThcD

■ database hits

YPIC Challenge

Team: Peptidal Investigators

Method:

Sample preparation

Dilution of peptide mixture in 0.1% TFA and preparation of aliquots corresponding to approx. 80 fmol per peptide

LC-MS/MS analysis

Ultimate 3000 nano RSLC system coupled to an Orbitrap Fusion Lumos mass spectrometer (both Thermo scientific)

100 μm x 2 cm pre-column / 75 μm x 50 cm C18 analytical column (PepMap RSLC , Thermo Scientific)
45 min gradient 3-50 % solvent B (solvent A: 0.1% FA, solvent B: 84% Acetonitrile, 0.1% FA)

Top speed acquisition (3 s) followed by creation of an inclusion list for subsequent runs

MS1 resolution: 120,000 @ 200 m/z

MS1 range: 300-1500 m/z

MS1 AGC target: 2.0e5

MS2 resolution: 30,000 @ 200 m/z

MS2 isolation window: 0.4 m/z

MS2 AGC target: 5.0e4

MS2 maximum injection time: 250 ms

MS2 Fragmentation: HCD (normalized collision energy 32%)

CID (collision energy 35%)

ETHcD (calibrated charge-dependent parameters for ETD, normalized collision energy 35% for supplemental activation)

Data analysis

PEAKS Studio 7.5 (<http://www.bioinfor.com>)

De novo peptide sequencing parameters:

Precursor mass tolerance: 10 ppm

Fragment ion mass tolerance: 0.02 Da

Enzyme specificity: none

Fixed modification: Carbamidomethylation (+57.01) (C)

Variable modifications: Oxidation (+15.99)(M)

Acetylation (+42.01)(K)

Methylation (+14.02)(R)

Phosphorylation (+79.96) (S)

Protection group (+89.97) (S)

Database search parameters (after identification of candidate sentence)

Search parameters mentioned as above

Database: *P. furiosus* entrapment database (2,045 entries, Uniprot, June 2017) + candidate sentence target-decoy search, filtering to 1% FDR on PSM level

Downstream analysis and inference of postulated sentence

www.google.com

Results:

I feel sure that there are many problems in chemistry which could be solved with far greater ease by this than by any other method. The method is surprisingly sensitive — more so even than that of spectrum analysis, requires an infinitesimal amount of material, and does not require this to be specially purified.

from the book

Rays of positive electricity and their application to chemical analyses

by **Joseph John Thomson**, published in 1913.