element | NANOSYN

BIO-ANALYTICAL ASSAYS OVERVIEW

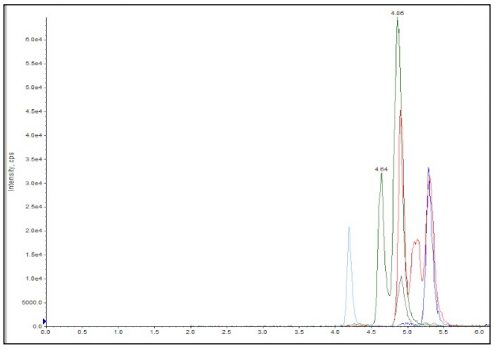
WE USE LCMS/MS/MS – THE HIGHEST SENSITIVITY MOLECULAR ANALYSIS

- Compound hydrolysis: quantification of hydrolysis product
- Covalent compound binding
- Lipidomics: quantify lipids produced by cells or in plasma, including phospholipids
- Proteomics: analyze proteins by individual peptides, including phospho-peptide mapping
- Metabolomics: quantify metabolites in specific metabolic pathways
- ADME kinetics: Absorption, distribution, metabolism, excretion

  Example studies:
  - Half-life and clearance in microsomes or hepatocytes
  - Permeability assay (with or without caco-2 cell bilayer)
  - Solubility assay
  - Protein binding rapid equilibrium dialysis (RED)
  - Compound stability in plasma or other media
- LC/MS/MS assay development

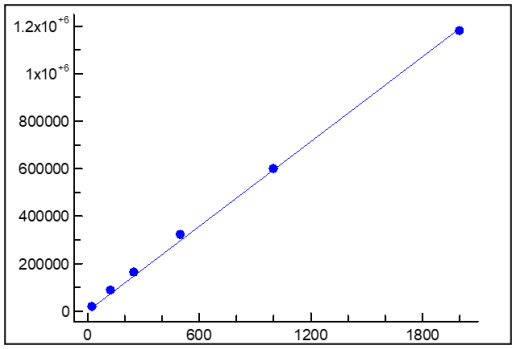
LCMS/MS/MS - SAMPLE PROJECT FOR LIPID ANALYSIS AT NANOSYN

- Intracellular phospholipids were separated and quantified with excellent linear correlation
- All 18 Acyl variants of PIPs were quantified at sub nmol levels on LCMS:
  - PI, PI3P, PI4P, PI5P, PI(3,4)PI2, PI(3,5)PI2, PI(4,5)PI2, PI(3,4,5)PI3



Lipid class
PI
PIP
PIP2
PI(4,5)P2
PIP3

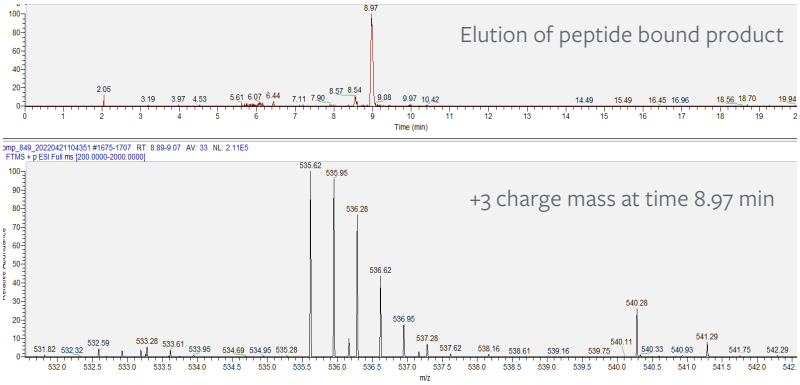
Example of all 8 phospholipid classes of 34:7 separated on chiral column in single injection



Example dynamic linear range for PI3P: 3 pMol to 300 pmol range

LCMS/MS - SAMPLE PROJECT FOR PEPTIDE BINDING AND COVALENCY AT NANOSYN

- Peptide sequencing of Kras G12C protein and identification of target peptide for binding of test compound after protein digest



KRAS G12C sequence confirmation using Proteome Discoverer software:

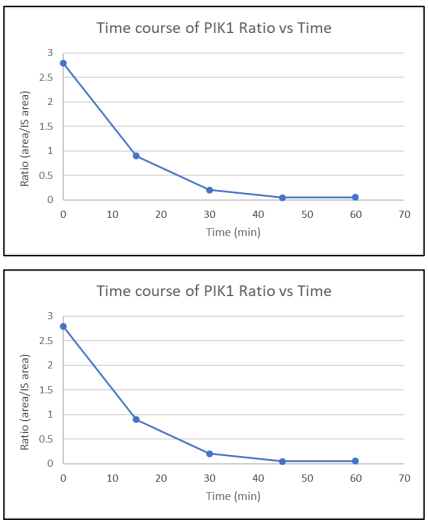
Table 2.

Description	Coverage [%]	# Unique Peptides	# AAs
GTPase KRas G12C	62%	17	189

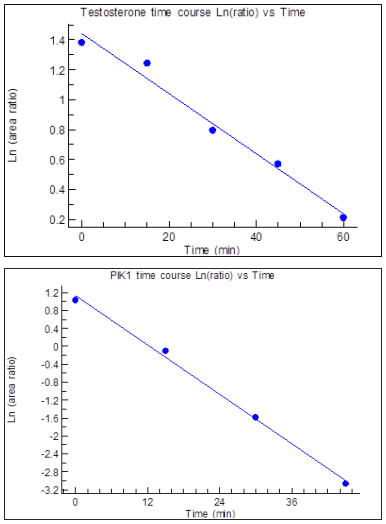
Sample spectra for covalent reference compound. +3 charge mass of peptide bound compound complex was found by LCMS. Original unbound peptide mass was not found in sample, therefore compound is fully covalent.

LCMS/MS - SAMPLE PROJECT FOR MICROSOME HALF-LIFE AT NANOSYN

- Test compounds were incubated with microsomes and disappearance was monitored over time by LCMS



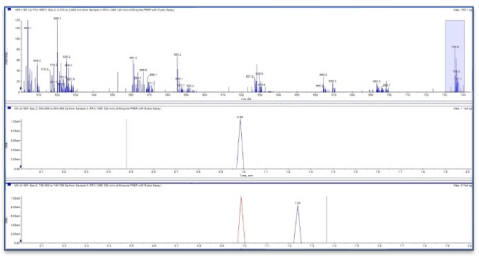
Time course of drug disappearance in microsome for testosterone and test compound.



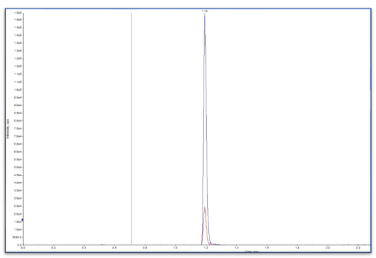
Natural log transform performed to calculate Half-life. Results showed nice linear relationship.

LCMS/MS - SAMPLE PROJECT FOR COMPOUND HYDROLYSIS AT NANOSYN

- Hydrolysis product and % loss of original mass was identified by LCMS after incubation of test compound with target enzyme



Hydrolysis mass and original mass both identified in the sample. % hydrolysis calculated based on ratio of peak areas.



Peak overlay of original intact mass at time 0 (blue) with time 120 min (red) of incubation of compound with enzyme.