



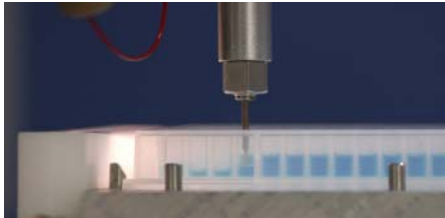
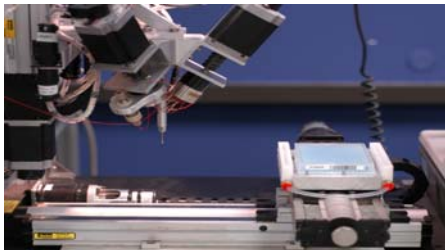
THE USE OF HIGH THROUGHPUT MASS SPECTROMETRY (HTMS) IN DRUG DISCOVERY

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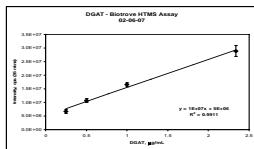
WHAT IS THE BIOTROVE HTMS?

- BioTrove's ultra High Throughput Mass Spectrometry (HTMS) system consists of a proprietary computer-controlled fluidic robot interfaced with a triple-quadrupole mass spectrometer. Robotics replace conventional HPLC (high performance liquid chromatography). The sample injection and purification system used is a modified LC-MS application.
- The BioTrove system can quantify multiple analytes simultaneously. The substrate and product of an assay can be quantified simultaneously, along with one or more internal standards as necessary.
- High quality data with coefficients of variability under 10% are routinely achieved in high-throughput screening applications. Z' values typically are > 0.6.
- The purification and mass spectrometry analysis steps described above are performed in a serial manner, eliminating the need for up-front sample preparation or purification. A wide range of buffers, salts, detergents, and compounds such as ATP or NADPH are fully compatible the BioTrove HTMS system.

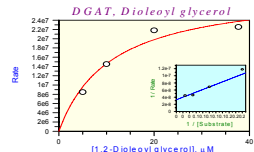
WHAT IS THE BIOTROVE HTMS: INSTRUMENTATION



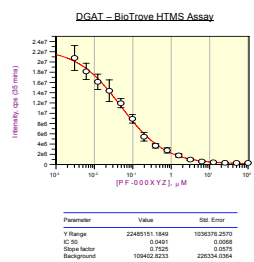
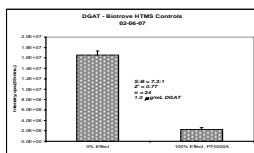
DGAT HTMS ASSAY DEVELOPMENT



There is a linear relationship between the amount of DGAT in the reaction and the amount of product formed.



The Km of 1,2-dioleoyl glycerol is 11 µM.



DGAT HTMS SCREENING WORKFLOW

513,180 compounds screened at 9 µM

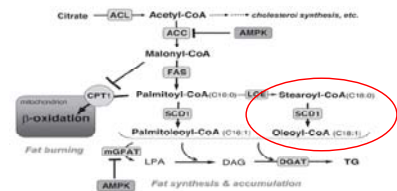
776 showed => 50% Inhibition (n=2), 0.15% hit rate

773 were available and were tested at 9 µM

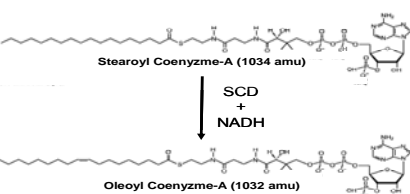
96 compounds showed => 40% Inhibition (n=3), 12.4% confirmation rate

50 actives demonstrated IC₅₀ <= 15 µM
14 of these actives <= 1 µM
Triaged actives were confirmed in cell assays

STEAROYL COA DESATURASE

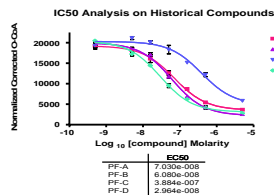


STEAROYL-COENZYME A DESATURASE



The BioTrove HTMS SCD assay is measuring a 2 amu difference between the desaturase substrate and product.

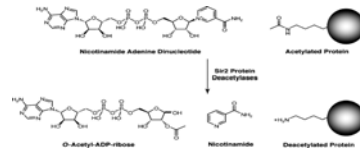
SCD HTMS HIT TO LEAD



The HTMS SCD assay easily differentiates various IC₅₀ potencies during hit to lead expansion.

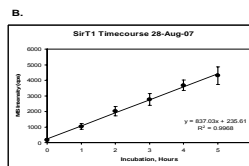
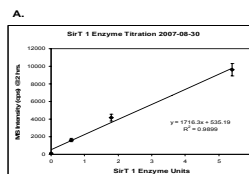
SIRT1 (HOMOLOG OF YEAST SILENT INFORMATION REGULATOR 2)

- Sirtuins are 'class III' NAD⁺ dependent histone deacetylases (HDACs)



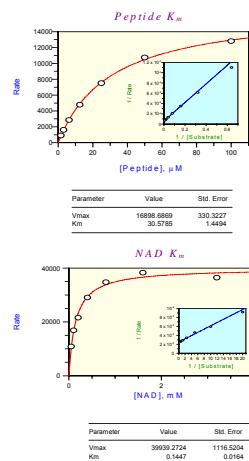
- NAD⁺ dependence links Sirt1 activity to metabolic status
- Up-regulated by caloric restriction (yeast, fly and rat); correlated to increased lifespan yeast, flies, worms and mice
- The Sirt1 activator Resveratrol extended lifespan in yeast, flies and mice
- Shown to be Sirt1 dependent in yeast and flies

Ac-EEKQGSTSSH-K(Ac)-Nle-STEGKEE-NH2



The BioTrove HTMS Sirt1 assay can detect changes in the deacetylation of a selected peptide. Figure A shows a linear relationship between the amount of product measured versus the amount of Sirt1 present in the reaction. Figure B indicates we are operating in a linear region of the reaction time.

Sirt1 HTMS Km DETERMINATIONS



AN EXAMPLE OF SAMPLE THROUGHPUT: DGAT

- 0.75 sec = sipper positioning into each well
- 6.25 sec = BioTrove analysis time
- 7 sec = total time per well
- 0.06 min = 3.6 sec = peak width
- 18 analysis points/peak
- 44.8 min = 384 wells
- 0.4 min = 24 sec = Wash time before each plate
- 0.8 min = 48 sec = Time between plates (Twister moving plates)
- 46 min = total time per plate
- 8 x 384-well plates/day = 4 plates/run (acquiring with Analyst software in Tune mode which freezes if running for too long)

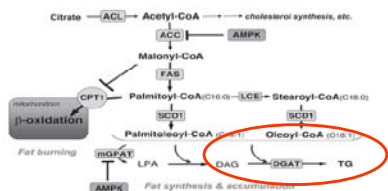
→ Although this is not high-throughput for HTS, it is high throughput for Mass Spec.
→ his technology may impact different areas of drug discovery due to its high data quality and higher throughput.

HOW ARE WE USING HIGH THROUGHPUT MS?

- Subset Screening for Acyl-CoA:Diacylglycerol Acyltransferase (DGAT)
 - Assay development
 - 210 plate compound mixtures subset screen
- Hit to Lead expansion with Stearoyl CoA Desaturase (SCD)
- Assay Development for Sirt1 deacetylase

SUBSET SCREENING: ACYL-COA:DIACYLGLYCEROL ACYLTRANSFERASE (DGAT) INHIBITION

DGAT catalyzes the terminal step of TG synthesis: transfer of acyl-CoA to diacylglycerol



SUMMARY

- The 1st generation BioTrove HTMS instrument was delivered to Pfizer Groton HTS Center of Emphasis in Sept. 2006.
- In 2007, the HTS CoE has applied the HTMS system to:
 - Assay development and Subset screening for DGAT
 - Hit to Lead exploration with Stearoyl CoA Desaturase (SCD)
 - Assay Development for Sirt1
- At this time in 2008, we have expanded our impact by supporting 7 drug targets at various discovery stages.

ACKNOWLEDGEMENTS

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