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STANFORD MASS SPECTROMETRY USERS' MEETING

September 2nd 2010
Dr. Maurizio Splendore



Qualitative Mass Spectrometry
for Elucidation
of Natural & Synthetic Products



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Overview

- General aspects of mass spectrometry
- Open Access Lab
- Example of Qualitative Analysis



Mass Spectrometry

- Ionization
- Ions are tractable
- Sensitivity
- Destructive analytical method
- Selective m/z (MS/MS)



Ionization

- The formation of ions is a critical part of every application of mass spectrometry
- Ion formation has to do with the energy required to produce an ion from a neutral molecule or the energy required to transfer pre-existing ions from a liquid or a solid phase into the gas phase
 - gas phase ion chemistry

Physical Review (1925-1930)



Ionization and Energy

- Molecular Ions



- Pseudo-Molecular Ions



- Odd electron ions

- Even electron ions



Ion Fragmentation

- Ion fragmentation has to do with the energy required to break particular chemical bonds that hold the ion together
- When the amount of energy transfer to the bond is controlled, specific bonds can be broken and molecular structural information can be obtained

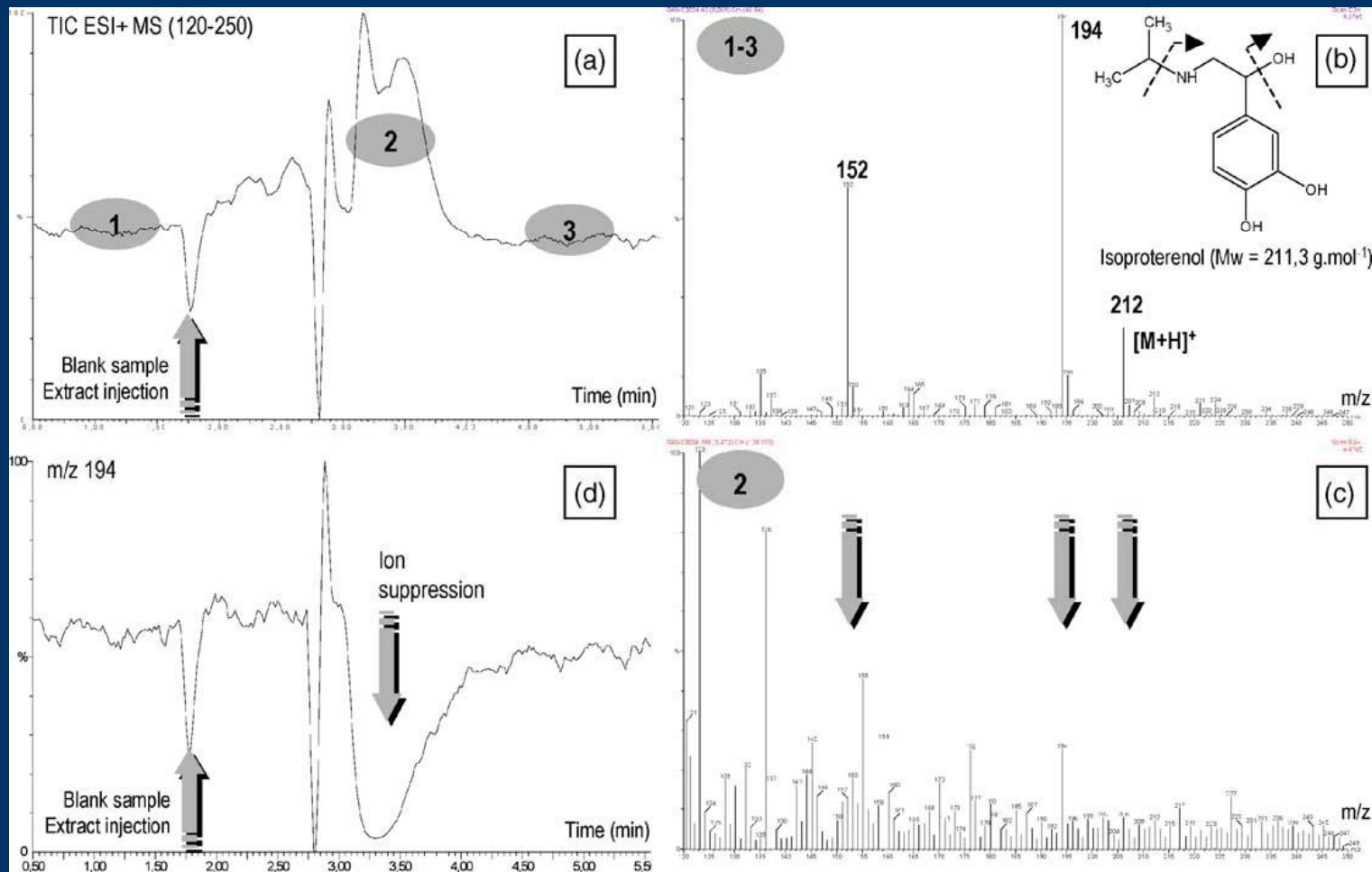


Ion Suppression

- Competition for either space or charge
 - Proton affinity
 - Surface tension
 - Viscosity
- Matrix effect
- Media of synthetic reactions
 - Reactants, products, co-factors

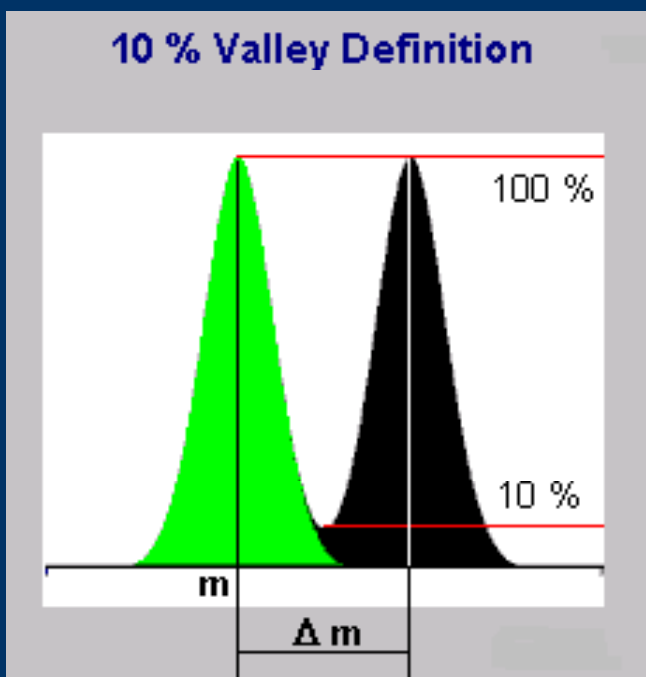


Ion suppression: Isoproterenol

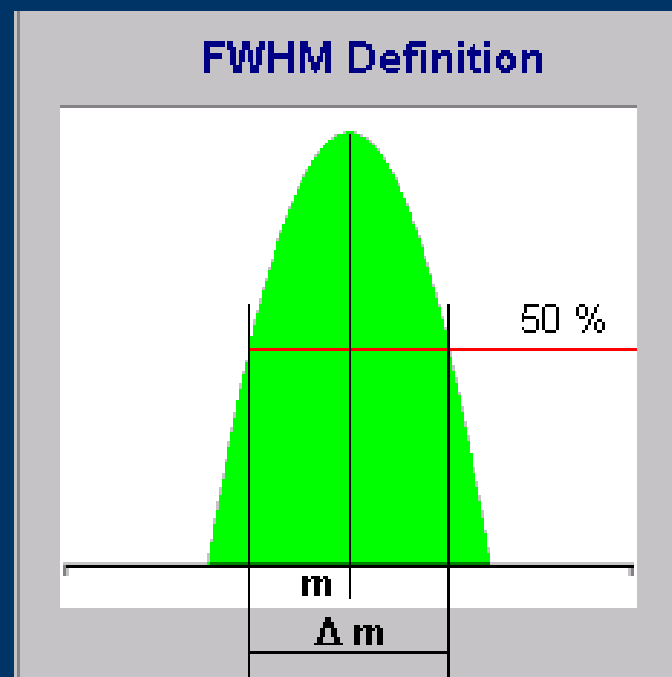




Mass Resolution and Accuracy



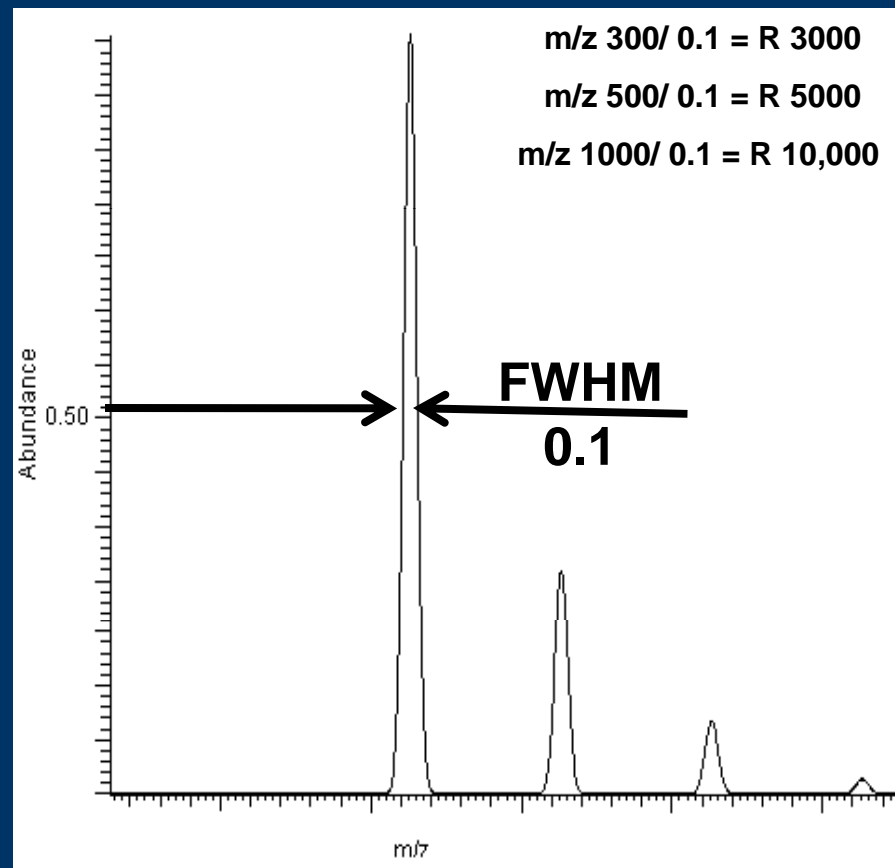
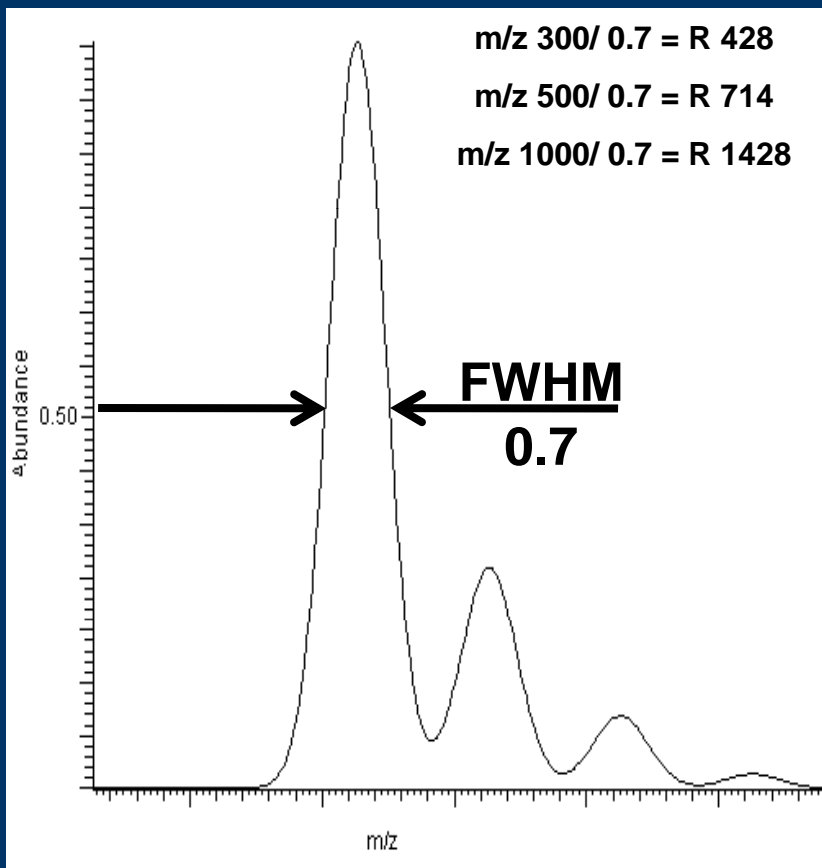
Magnetic Sector:
Resolution is constant with mass
Peak width varies with mass
10% Valley Definition



Quadrupole, Ion Trap, TOF:
Resolution varies with Mass
Peak width constant with Mass
Full Width Half Mass Definition

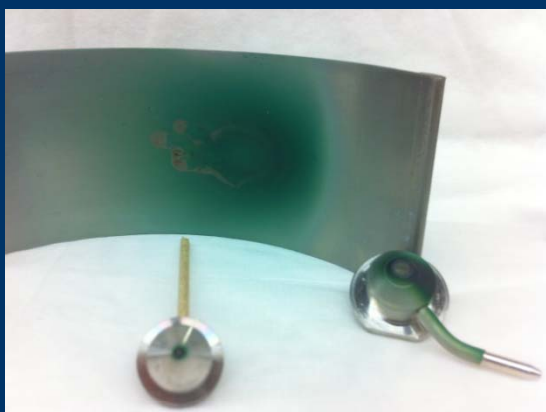


Resolution FWHM





MS is a Destructive Method





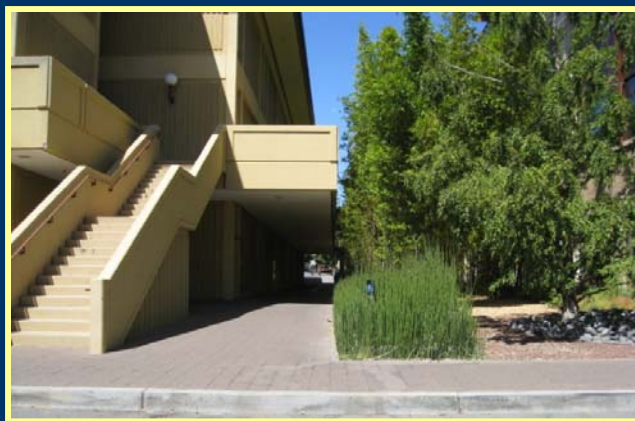
Open Access Lab





SUMS

- Open Access Lab: Mudd Bldg, room 175A
- 200 MS Users
- Username `user@stanford.edu`
- SUID # Stanford ID card #



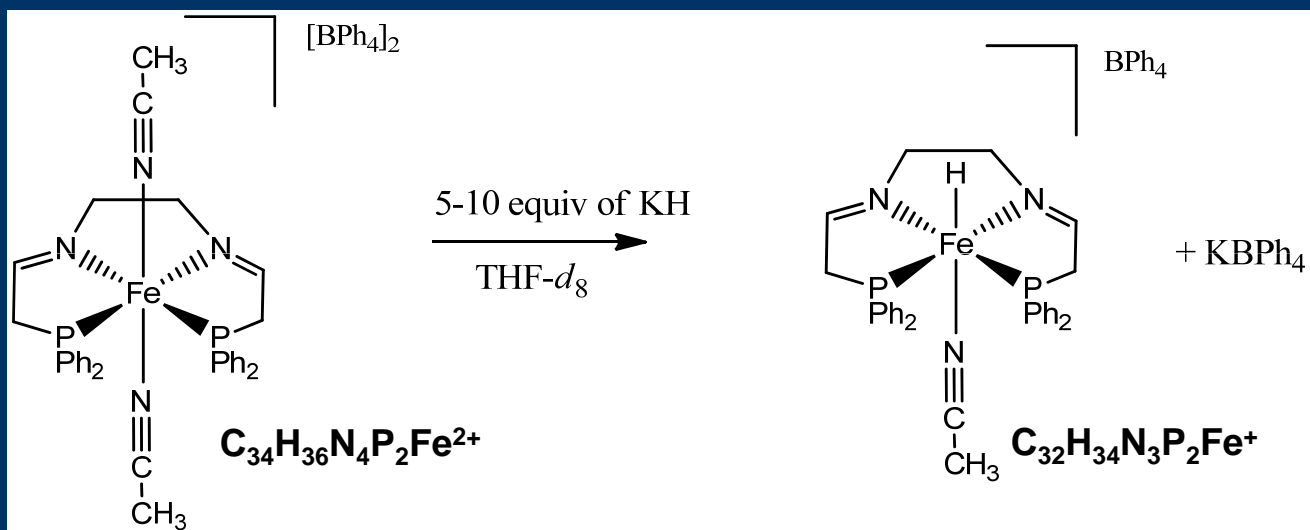
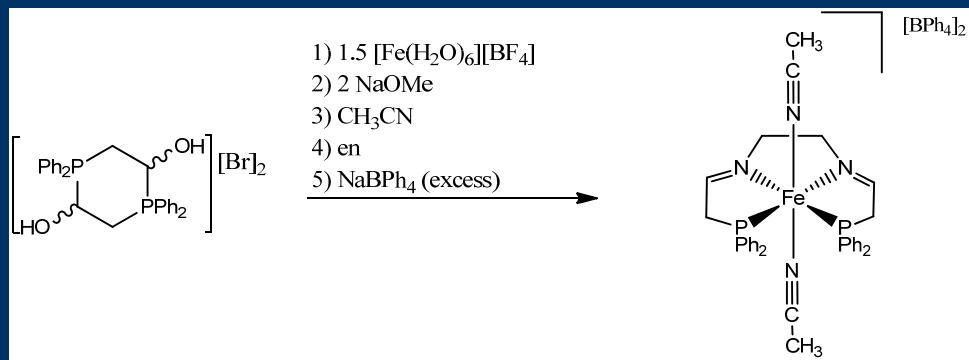


Open Access Lab

- GC-MS (EI)
 - Non Polar Compounds
 - Mass Range 50-550 Da
- LC-MS (ESI)
 - Polar Compounds
 - Mass Range 50-2000 m/z
- DESI-MS/MS
 - Polar Compounds and Solids
 - Mass Range 50-2000 m/z



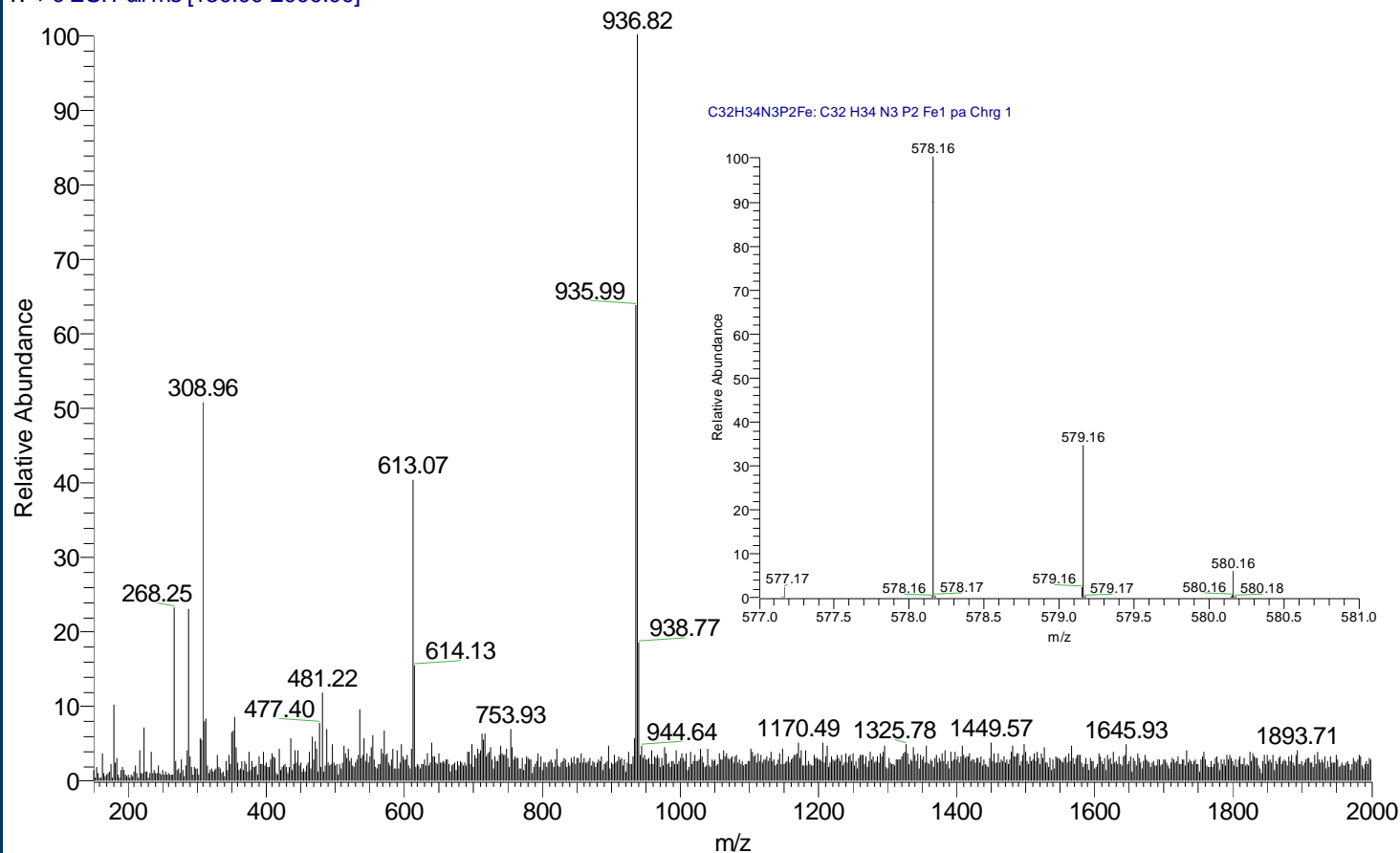
Organometallic Compound





DESI Mass Spectrum

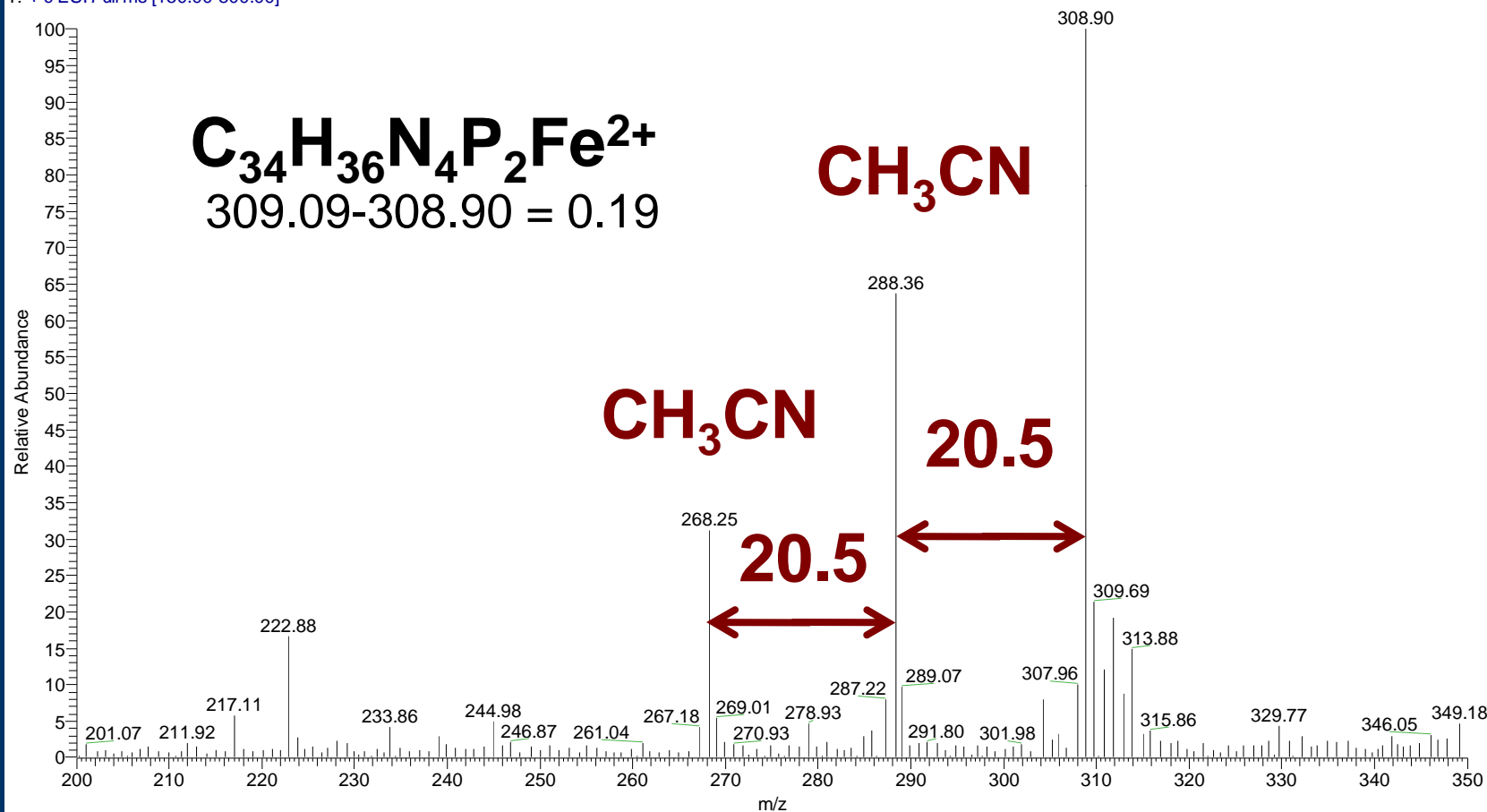
Decrisci100603_23914 #70-104 RT: 1.22-1.96 AV: 29 NL: 7.08E6
T: + c ESI Full ms [150.00-2000.00]





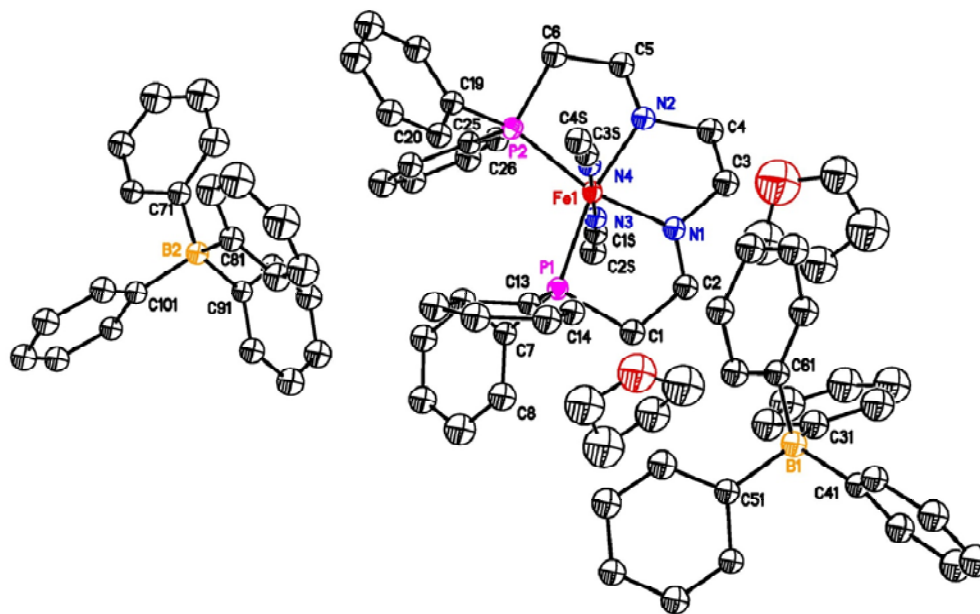
DESI Mass Spectrum

Decrisci100603_23914 #17-107 RT: 0.51-1.21 AV: 59 NL: 6.71E6
T: + c ESI Full ms [150.00-800.00]



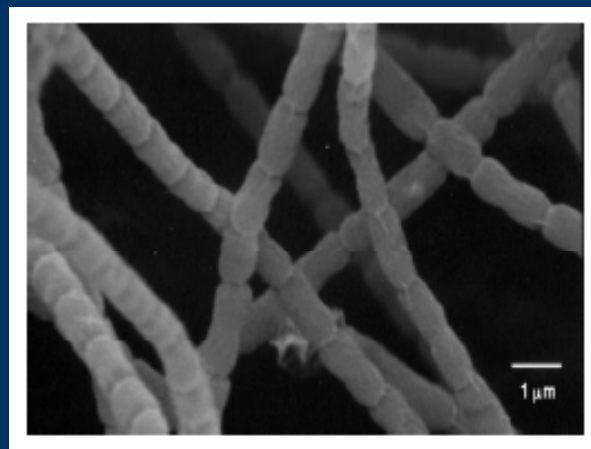


X-ray Crystallography





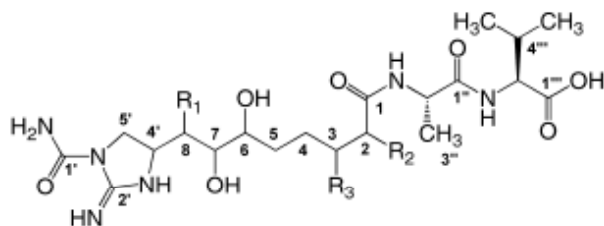
Enteropathogenic *Escherichia coli* expressing the Type II secretion system induced hemolysis of sheep blood cells



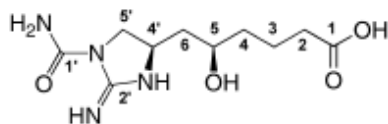
Streptomyces sp. K01-0509



Guadinomine



	R ₁	R ₂	R ₃
Guadinomine A (1)	OH	NH ₂	NH ₂
Guadinomine B (2)	H	NH ₂	NH ₂
Guadinomines C ₁ (3) and C ₂ (4) (diastereomers at C-6')	H		
Guadinomine D (5)	H		NH ₂



Guadinomic acid (6)
(K01-0509 B)



Resistant Antibiotics

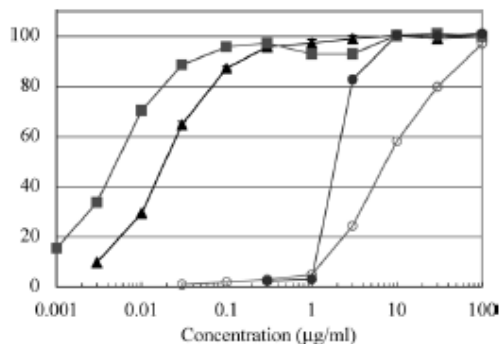
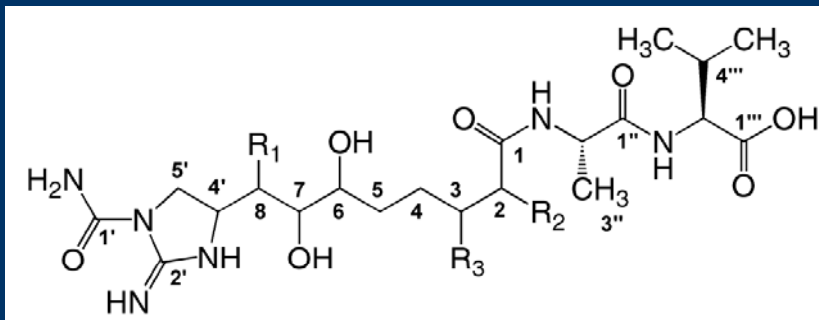


Fig. 7 Inhibitory activity of gudinomines against Type III secretion system of EPEC.

Gud. A (▲), B (■) and D (○) and tetracycline (●).

Guad	MW (g/mol)	Yield (mg/L)	IC ₅₀ (µg/mL)
A	518	0.66	0.02
B	502	1.1	0.007
C	556	0.025	0
D	544	4.4	8.5
Acid	258	1	0



Omura J. Antibiot. 61(4)222, 2008



+/- ESI Mass Spectra Guadinomine A

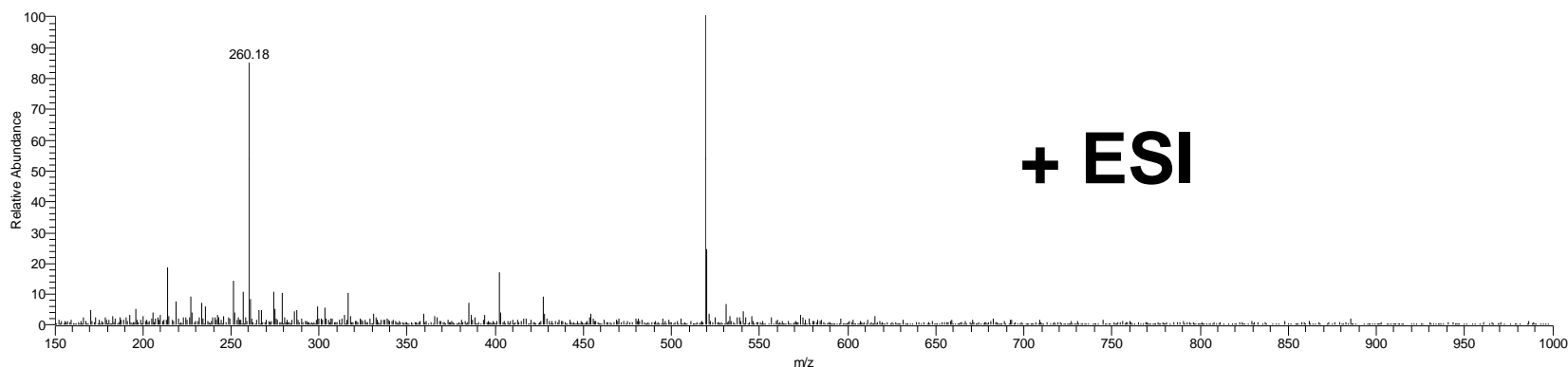
C:\Xcalibur\...\HolmesT_100310_22408_Gua

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Guadinomine

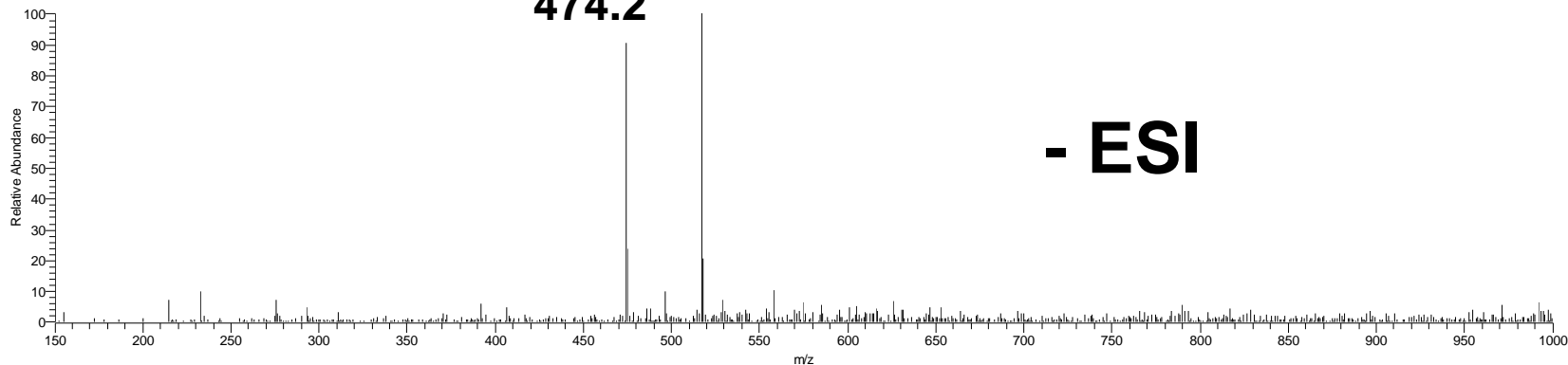
HolmesT_100310_22408_Gua #38-52 RT: 1.08-1.41 AV: 7 NL: 4.87E6
F: + c ESI Full ms [150.00-1000.00]

519.2



HolmesT_100310_22408_Gua #38-53 RT: 1.05-1.44 AV: 8 NL: 1.37E6
F: - c ESI Full ms [150.00-1000.00]

474.2 516.9





Biosynthesis of Guadinomines

- R5 medium, 1 L liquid culture, 30°C shaker, 4 days, aerobic conditions
 - 0.3M Sucrose, 1.4mM K_2SO_4 , 50mM $MgCl_2 \cdot 6H_2O$
 - 55mM Glucose, 25mM TES Free Acid (Buffer), 2mL Trace Elements Solution
 - 4mL 5M $CaCl_2 \cdot 2H_2O$, 7mL 20% L-Proline
 - 10mL 0.5% KH_2PO_4 , 7mL 1M NaOH
 - Sterilize medium by autoclaving
- Extract with 1 L Acetone (acetone lyses the cells)
 - Rotovap acetone, leaving 1 mL crude aqueous extract
 - Filter & submit for Mass Spec



Extract Analysis

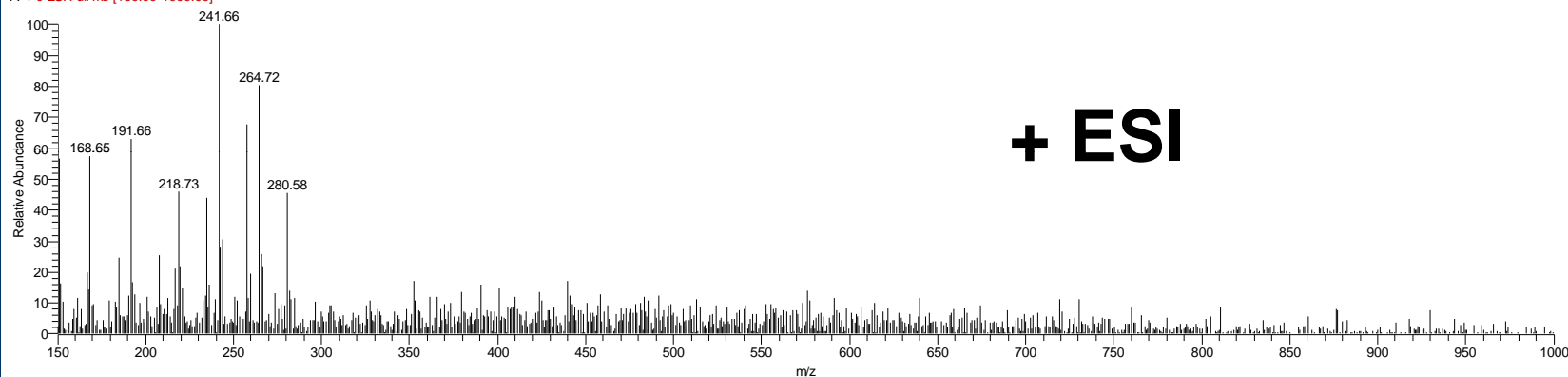
C:\Xcalibur\data\HolmesT_100608_23914_06

6/8/2010 4:11:41 PM

R5 Crude 1

HolmesT_100608_23914_06 #91-105 RT: 3.31-3.71 AV: 8 NL: 5.89E5

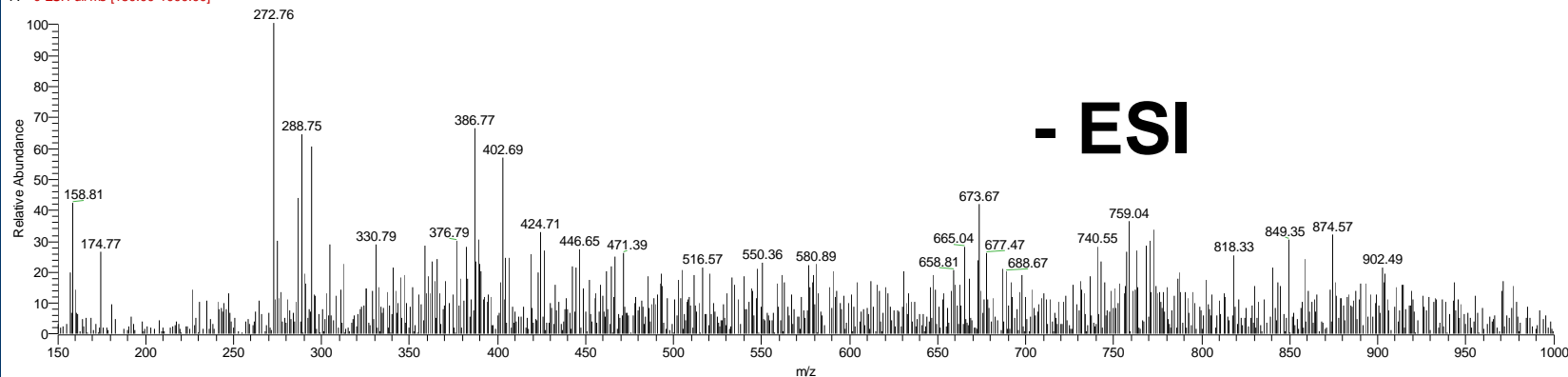
F: + c ESI Full ms [150.00-1000.00]



+ ESI

HolmesT_100608_23914_06 #89-107 RT: 3.28-3.74 AV: 9 NL: 1.26E5

F: - c ESI Full ms [150.00-1000.00]



- ESI



Improved sample prep

- DOWEX 50W x2 [H⁺] Ion Exchange Column
 - Strong Cation Exchanger
 - Elute with 1.5N NH₄OH
 - Neutralize with conc. HCl
 - Lyophilize and resuspend in minimal amount of H₂O
 - Filter & submit for Mass Spec



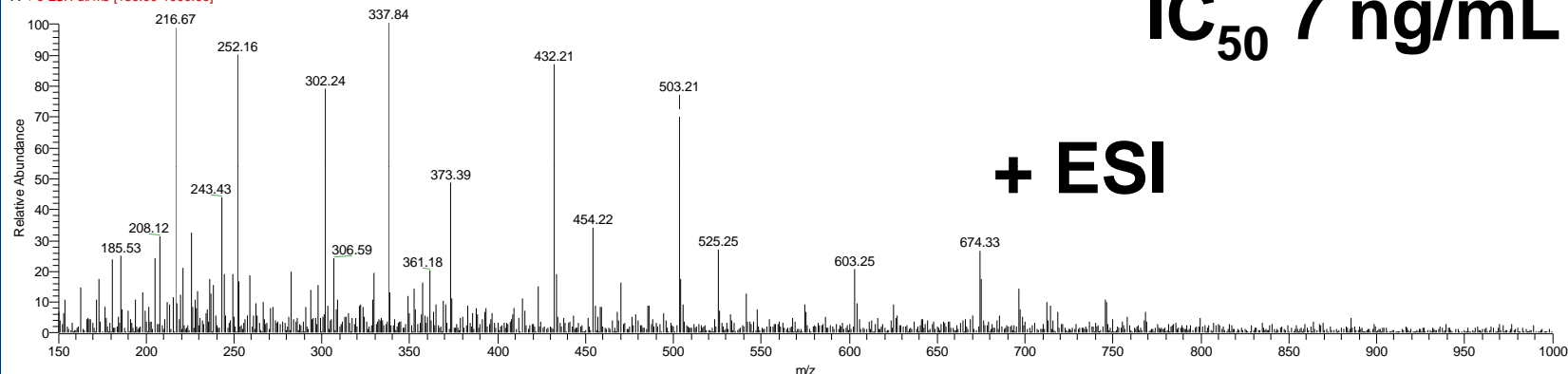
Guadinomine B: +/- ESI Mass Spectra

C:\Xcalibur\data\HolmesT_100608_23914_01

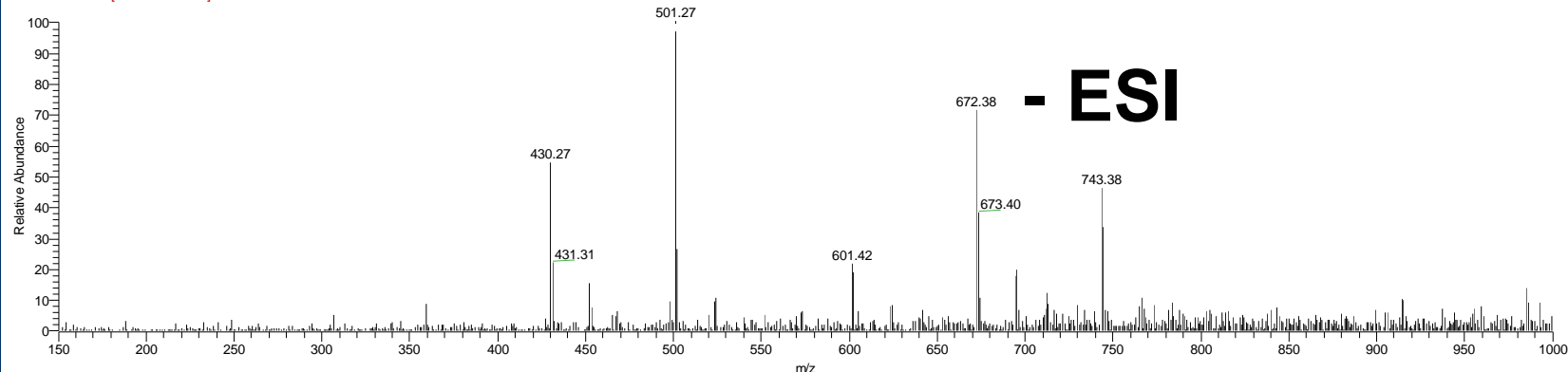
6/8/2010 2:00:01 PM

R5 IEC 1

HolmesT_100608_23914_01 #99-130 RT: 3.20-4.03 AV: 16 NL: 1.31E6
F: + c ESI Full ms [150.00-1000.00]



HolmesT_100608_23914_01 #99-138 RT: 3.23-4.30 AV: 20 NL: 6.67E5
F: - c ESI Full ms [150.00-1000.00]





Acknowledgements

- Allis Chien
- Karolina Krasinska
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