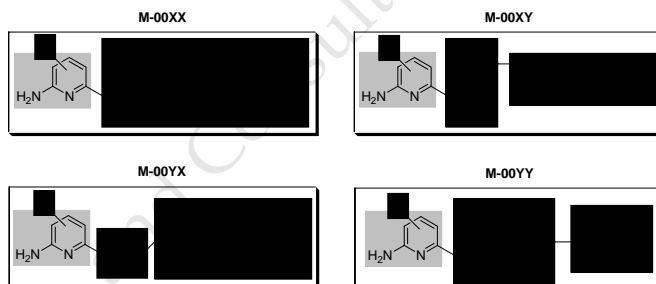


M-00XX_XY_YX_YY RLM rMetID Report



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Protocol, Instrumentation, and Conditions



Project Info

Compound ID	M-00XX_YY	Study Level	rMetID	Quotation ID	\	Request Date	3/5/2010	Experiment Date	3/27-3/29
Requested by	\	Requested Studies	RLM	Cmpd Record	\	Cmpd Received	3/24/2010	Report Date	3/31/2010

Incubation and Sample Preparation Protocol

- Concentration of in vitro incubations (all concentrations are final):

Biological matrices	RLM	1.0 mg/mL	Solvent	KPi buffer, pH 7.4	100 mM	GSH	5.0 mM
Test Compound	M-00XX_YY	50.0 μ M	Cofactor	NADPH	1.0 mM	Time/Temp.	60 min @ 37 $^{\circ}$ C

- Control is made by replacing NADPH with KPi buffer; total volume of each incubation is 200 μ L;

- Incubations were quenched with 30 μ L of TCA (10%) solution followed by centrifuge;

- Supernatant was then analyzed by LC-PDA-MS/MS.

LC-PDA-MS/MS Instrumentation

- Thermo Finnigan Surveyor HPLC (auto-sampler, MS pump, PDA detector) *tandem* Thermo Finnigan LCQ Ion Trap Mass Spectrometer (ITMS, low resolution)

LC-PDA-MS/MS Analytical Conditions

LC-PDA	ITMS
Solvents	Mode
A: H ₂ O (1% NH ₄ OAc, pH=10); B: MeOH (1% NH ₄ OAc, pH=10)	ESI, Positive
Flow	Spray Voltage
750 μ L/min	4.5 kV
PDA	Capillary Temperature
230-400 nm	225 $^{\circ}$ C
M-00XXYYX	Capillary Voltage
Column	20 V
THERMO Hypersil BiBasic-4, 50x3 mm, 5 μ , #46	Sheath Gas (N ₂)
Program	40 (arbitrary unit)
B%: 0-5 min, 20; 5-15 min, 20 to 40; 15-35 min, 40 to 60; 35-38 min, 60 to 90; 38-40 min, 90 to 20.	Collision Energy
M-00XYYY	45 eV
Column	
Phenomenex Prodigy ODS3, 100x3.2 mm, 5 μ , #43	
Program	
B%: 0-5 min, 20; 5-15 min, 20 to 40; 15-30 min, 40 to 60; 30-33 min, 60 to 90; 33-35 min, 90 to 20; 35-36 min, 20.	

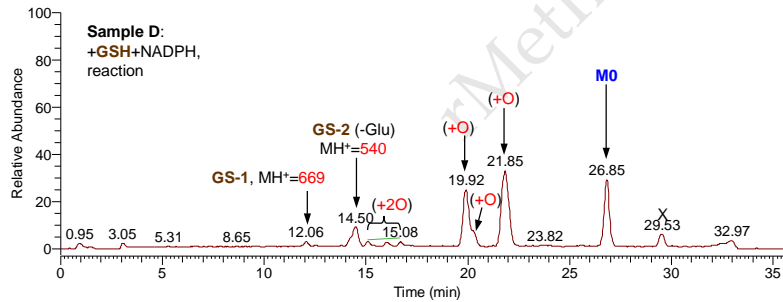
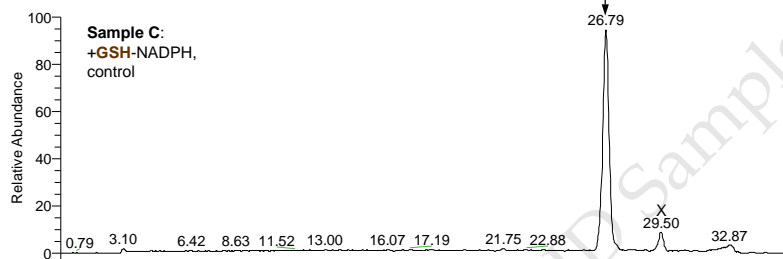
Reactive/Toxic Metabolite In Vitro Screening Results

Cmpd	Structure	MH ⁺ (MO)	GSH Adduct(s) Detection	MH ⁺ (GSH Adduct)	Bioactivation Equation
M-00XX		373	Negative	\	\
M-00XY		409	Negative	\	\
M-00YZ		372	Negative	\	\
M-00YY		348	Positive	669 540 (-Glu)	Adduct = [MO + O] + GS

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LC-MS Analysis of M-00YY RLM-GSH Incubation Samples

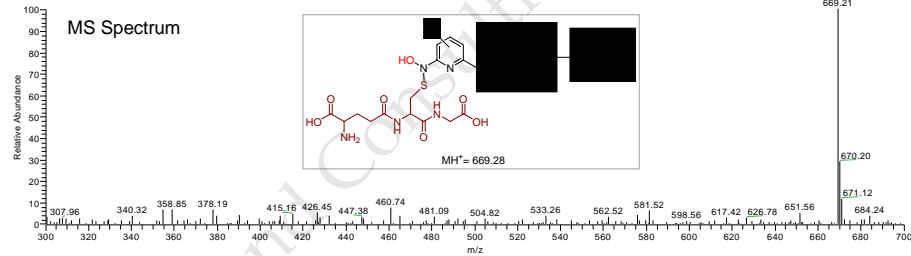
RT: 0.00 - 35.99 SM: 15G



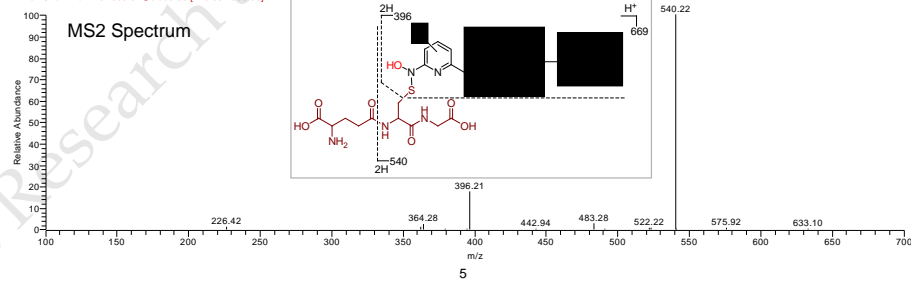
4

GS-1 (+O+GS), MH⁺=669, in M-00YY RLM-GSH Incubation

15RD-50-GSH-5-37-60-200-30-0501-A4Bp18c43-300-700-4-ms2-20 #957-1003 RT: 11.53-12.27 AV: 35 SB: 106 10.32-10.97, 13.38-14.75 NL: 8.33E4
T: + c ESI d w Full ms2 669.31@cid50.00 [170.00-1350.00]

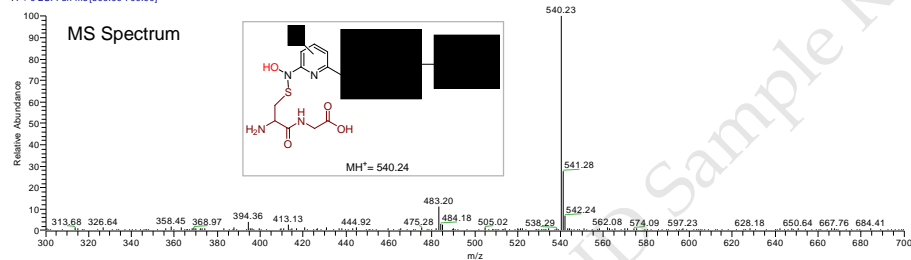


15RD-50-GSH-5-37-60-200-30-0501-A4Bp18c43-300-700-4-ms2-20 #826-1117 RT: 11.84-12.24 AV: 9 NL: 8.49E4
F: + c ESI d w Full ms2 669.31@cid50.00 [170.00-1350.00]

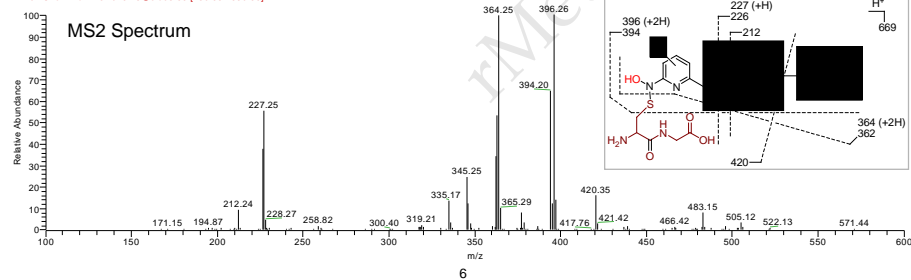


GS-2 (+O+GS-Glu), MH⁺=540, M-00YY RLM-GSH Incubation

15RD-50-GSH-5-37-60-200-30-0501-A4Bp18c43-300-700-4-ms2-20 #1119-1152 RT: 14.09-14.82 AV: 9 SB: 48 13.57-13.78, 15.54-16.87 NL: 7.89E5
T: + c ESI d w Full ms2 540.19@cid50.00 [135.00-1095.00]



15RD-50-GSH-5-37-60-200-30-0501-A4Bp18c43-300-700-4-ms2-20 #1048-1217 RT: 13.04-14.97 AV: 14 NL: 7.82E4
F: + c ESI d w Full ms2 540.19@cid50.00 [135.00-1095.00]



Proposed Bioactivation Pathway of M-00YY in RLM

