

M-00XX RLM MetID Report



Beijing | Chicago

MetID Contract Research | ADME-MedChem Outsourcing Consulting |
Drug Discovery Methodology Research
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Project Info, Protocol, Instrumentation, and Conditions



Project Info

Compound ID	M-00XX	Study Level		MetID-III		Quotation ID	\	Request Date	7/20/2009	Experiment Date	7/24-7/25
Requested by	\	Requested Studies	RLM	Compd Record	3.80 - 2.60 = 1.20 mg	Compd Received	7/24/2009	Report Date	7/26/2009		

Incubation and Sample Preparation Protocol

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

Biological matrices	LM	1.0 mg/mL	Solvent	KPi buffer, pH 7.4	100 mM	DMSO%	0.2
Test Compound	M-00XX	20.0 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 equal volume of MeOH/CH₃CN (1/4, v/v) followed by centrifuge;
- Supernatant was then analyzed by LC-PDA-MS/MS.

LC-PDA-MS/MS Instrumentation

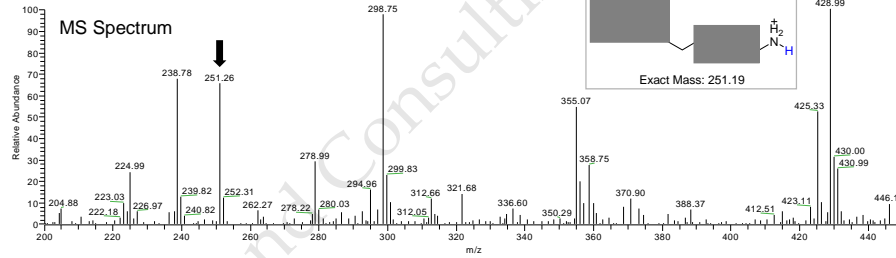
- Thermo Finnigan Surveyor HPLC (auto-sampler, MS pump, PDA detector) *tandem* Thermo Finnigan LCQ Ion Trap Mass Spectrometer (ITMS, low resolution)

LC-PDA-MS/MS Analytical Conditions

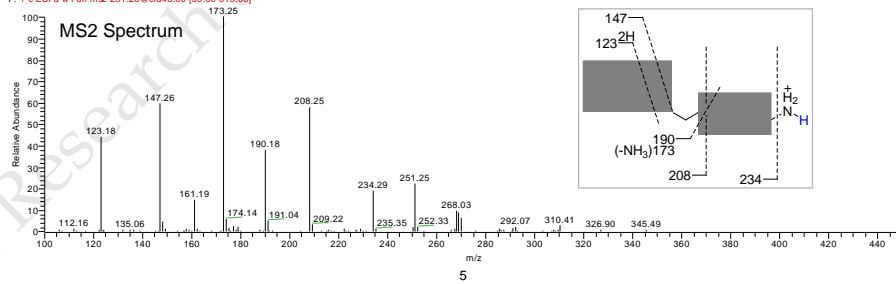
LC-PDA	ITMS
Column	Mode
THERMO Kromasil C18, 50x3 mm, 5 μ , #53	ESI, Positive
Solvents	Spray Voltage
A: H ₂ O (1% NH ₄ Ac, pH 9.0); B: MeOH (1% NH ₄ Ac, pH 9.0)	4.5 kV
Flow	Capillary Temperature
750 μ L/min	225 $^{\circ}$ C
PDA	Capillary Voltage
200-400 nm	20 V
Program	Sheath Gas (N ₂)
B%: 0-2 min, 20; 2-8 min, 20 to 40; 8-16 min, 40-70; 16-19 min, 70 to 20; 19-20 min, 20.	40 (arbitrary unit)
	Collision Energy
	50 eV

M1 ($C_8H_5F_3$), $MH^+=251$

13B-20-37-60-200-30-0724-230a4d1c1c46-200-450-4-m2 #572-634 RT: 7.22-8.67 AV: 13 SB: 60 6.10-8.01, 10.97-12.98 NL: 4.27E5
T: + c ESI Full ms [200.00-450.00]

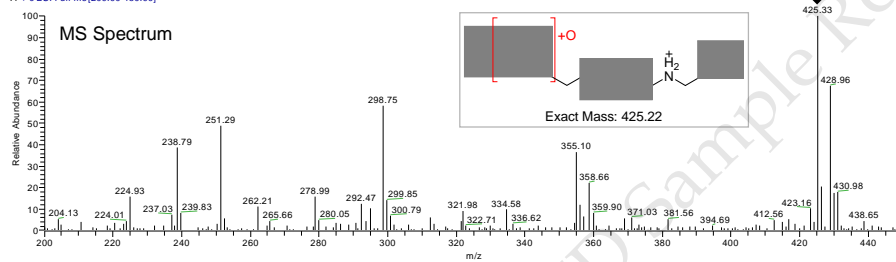


13B-20-37-60-200-30-0724-230a4d1c1c46-200-450-4-m2 #563-648 RT: 7.39-8.85 AV: 13 NL: 6.06E4
F: + c ESI d w Full ms2 251.26@cid45.00 [55.00-515.00]

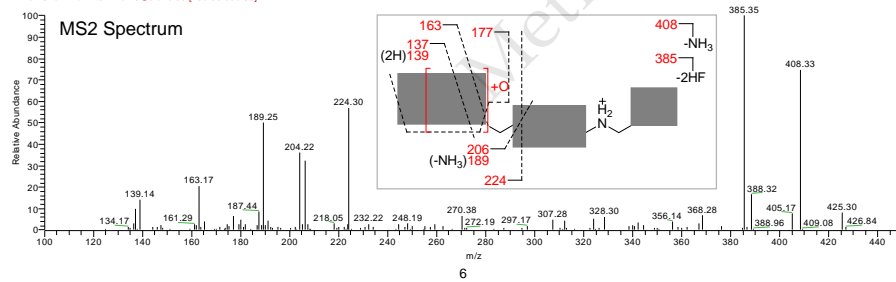


M2, (+O) $MH^+=425$

13B-20-37-60-200-30-0724-230a4d1c1c46-200-450-4-m2 #615-629 RT: 8.30-8.55 AV: 3 SB: 48 5.36-7.29, 10.36-11.48 NL: 8.05E5
T: + c ESI Full ms [200.00-450.00]



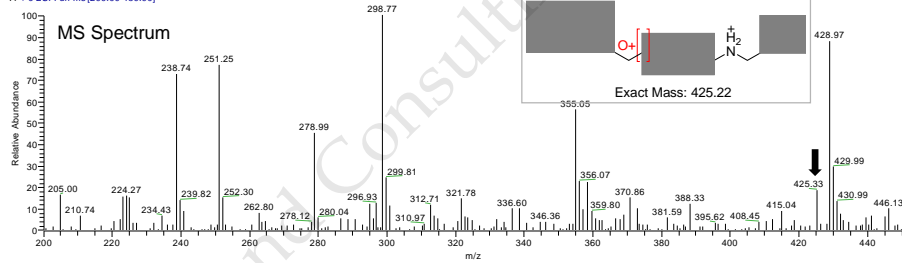
13B-20-37-60-200-30-0724-230a4d1c1c46-200-450-4-m2 #605-648 RT: 8.32-8.71 AV: 4 NL: 1.21E5
F: + c ESI d w Full ms2 425.29@cid45.00 [105.00-865.00]



M3, (+O) MH⁺=425

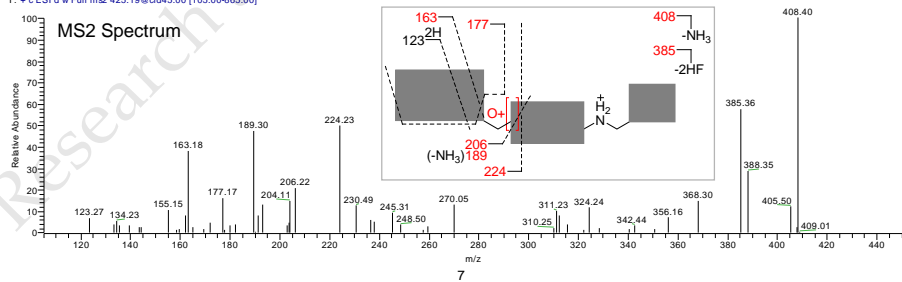
138-20-37-60-200-30-0724-230a4dc1c46-200-450-4-ms2 #590-604 RT: 7.70-7.94 AV: 3 SB: 77 5.10-7.44, 11.85-13.66 NL: 4.69E5
T: + c ESI d w Full ms [200.00-450.00]

MS Spectrum



138-20-37-60-200-30-0724-230a4dc1c46-SIM-267-425-ms2 #293 RT: 7.86 AV: 1 SB: 54 5.35-7.29, 10.36-11.49 NL: 6.95E4
T: + c ESI d w Full ms2 425.19@cd45.00 [105.00-865.00]

MS2 Spectrum



Proposed Metabolism Pathway of M-00XX in RLM

