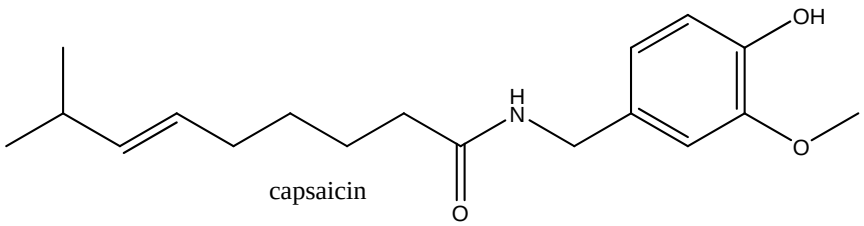
S1 Supporting Information Contents Page

Title: Determination of Analyte Concentration using the Residual Solvent Resonance in ^{1}H NMR Spectroscopy

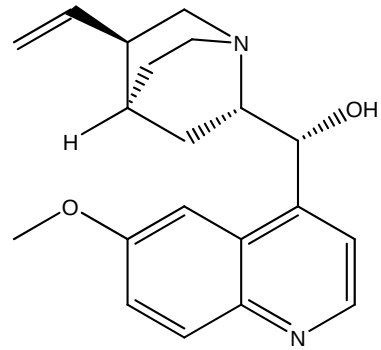
Authors: Gregory K. Pierens, Anthony R. Carroll, Rohan A. Davis, Meredith E. Palframan, and Ronald J. Quinn

Contents:

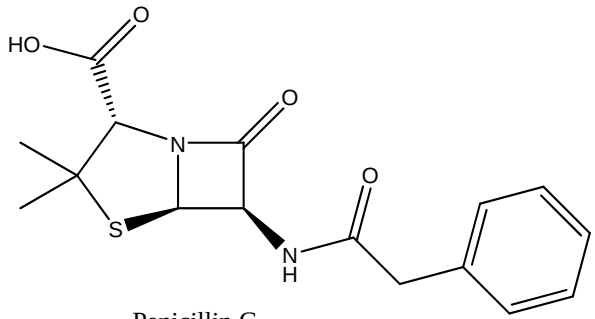
S2 Chemical Structures of the Commercially Available Crystalline Compounds (neutral form)



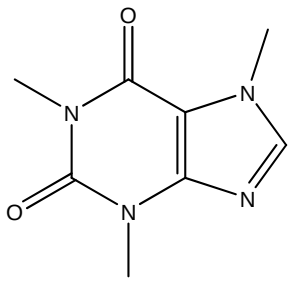
capsaicin



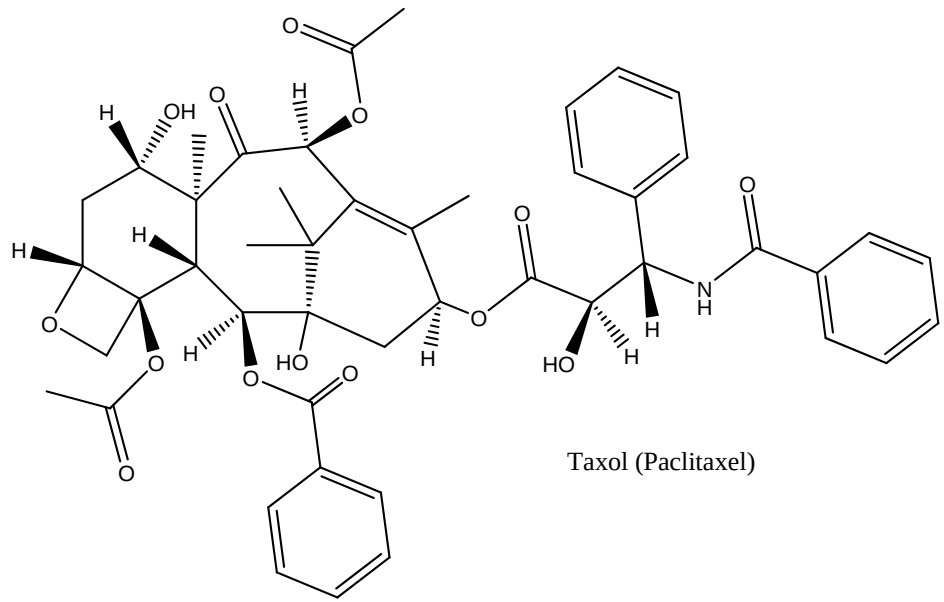
quinine



Penicillin G



caffeine



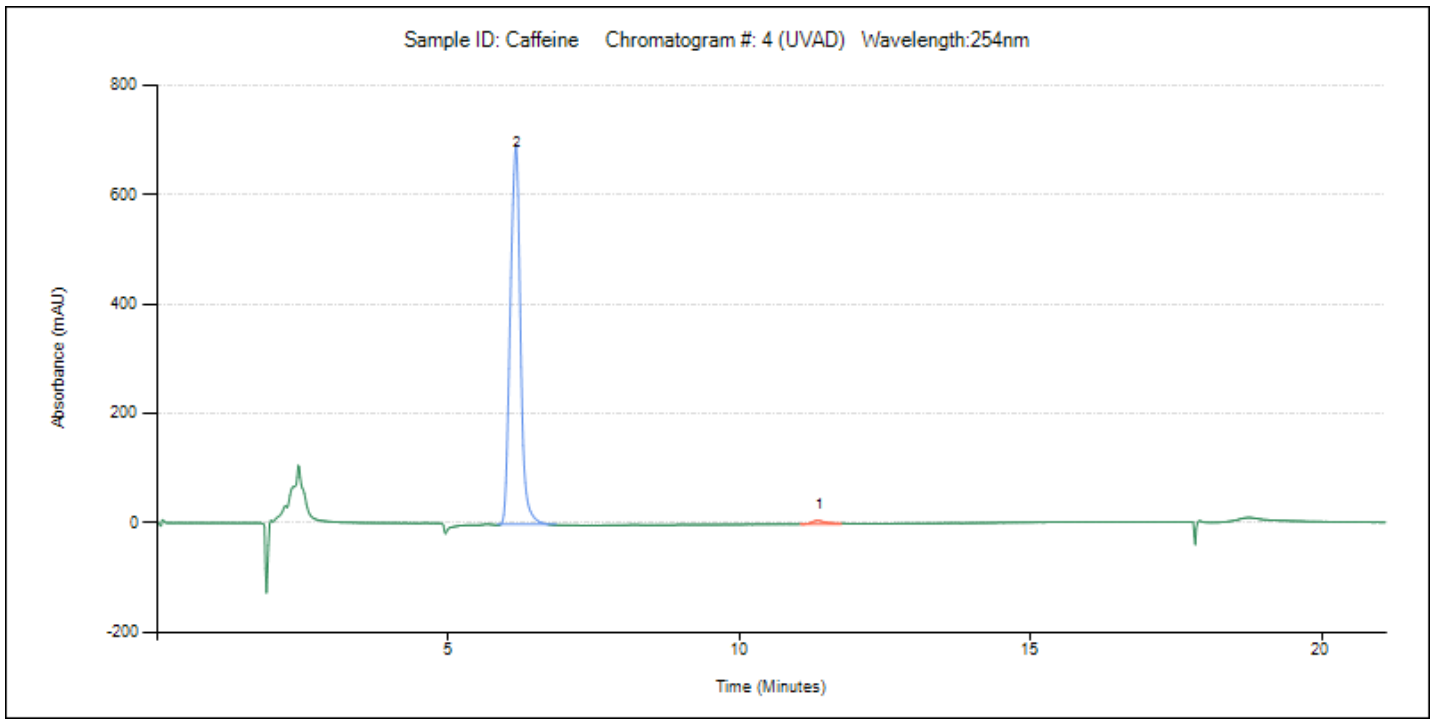
Taxol (Paclitaxel)

S3 Example of C_{18} μPLC Purity Analysis (Caffeine)

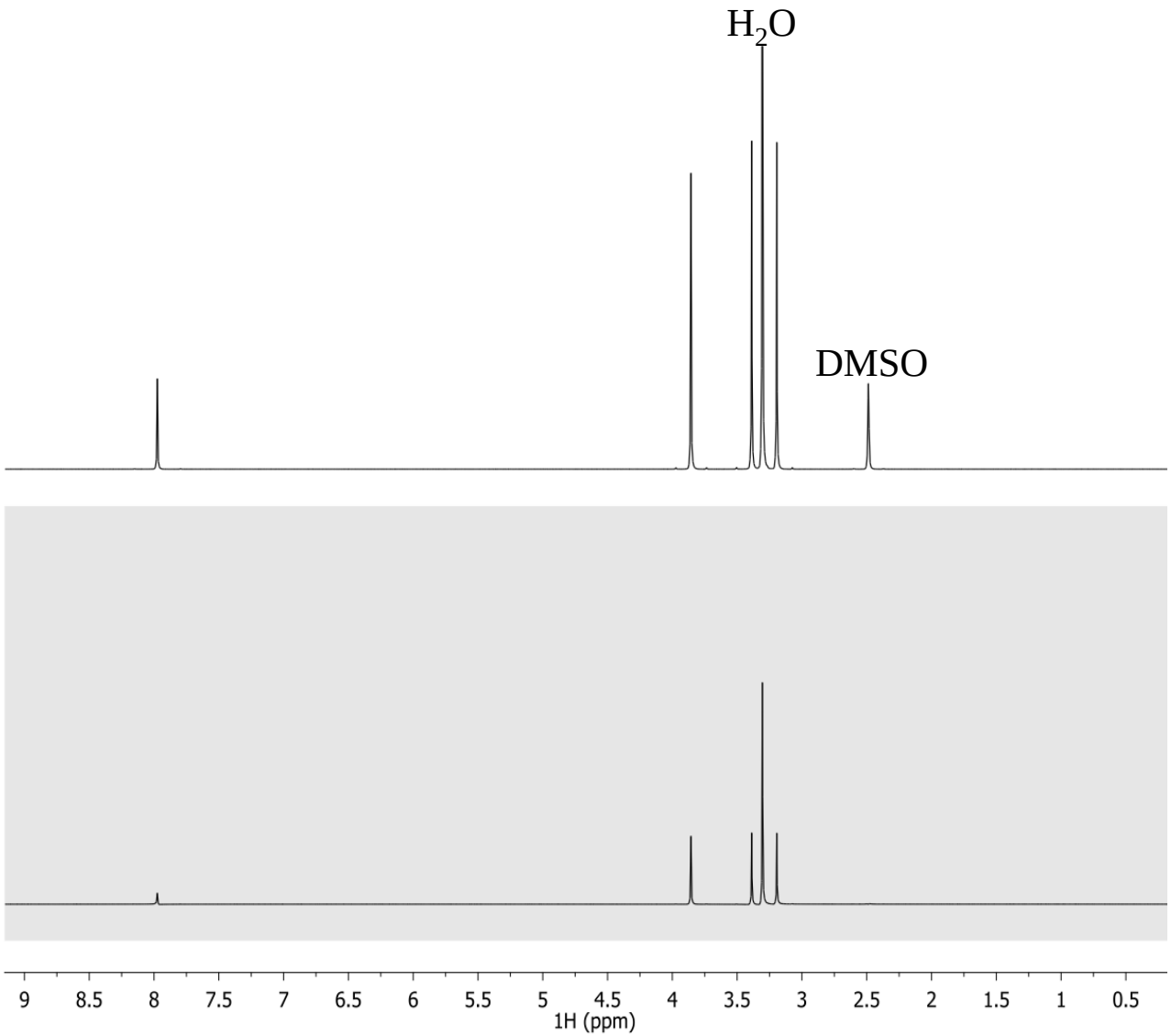
Data File: COMPOUND PURITY ANALYSIS-002.dat
Method File: Pure Compound Analysis
Column No.: 4
Wavelength (nm): 254
Sample ID: Caffeine
Load Volume: 3 μL
Column Type: 4209002 C_{18} 7 μm

Peak Information

Peak #	*Retention Time*	*Area*	*Area %*	*Height*	*FWHM*
1	11.25	1.54	1.08	6.19	0.23
2	6.08	140.89	98.92	693.47	0.19



S4 Example of Two Step qNMR Analysis in DMSO-d_6 (Caffeine)



Step 2: 1H NMR spectrum with d1= 60 s. Positively phased signals from step 1 can be used for integration and concentration determination.

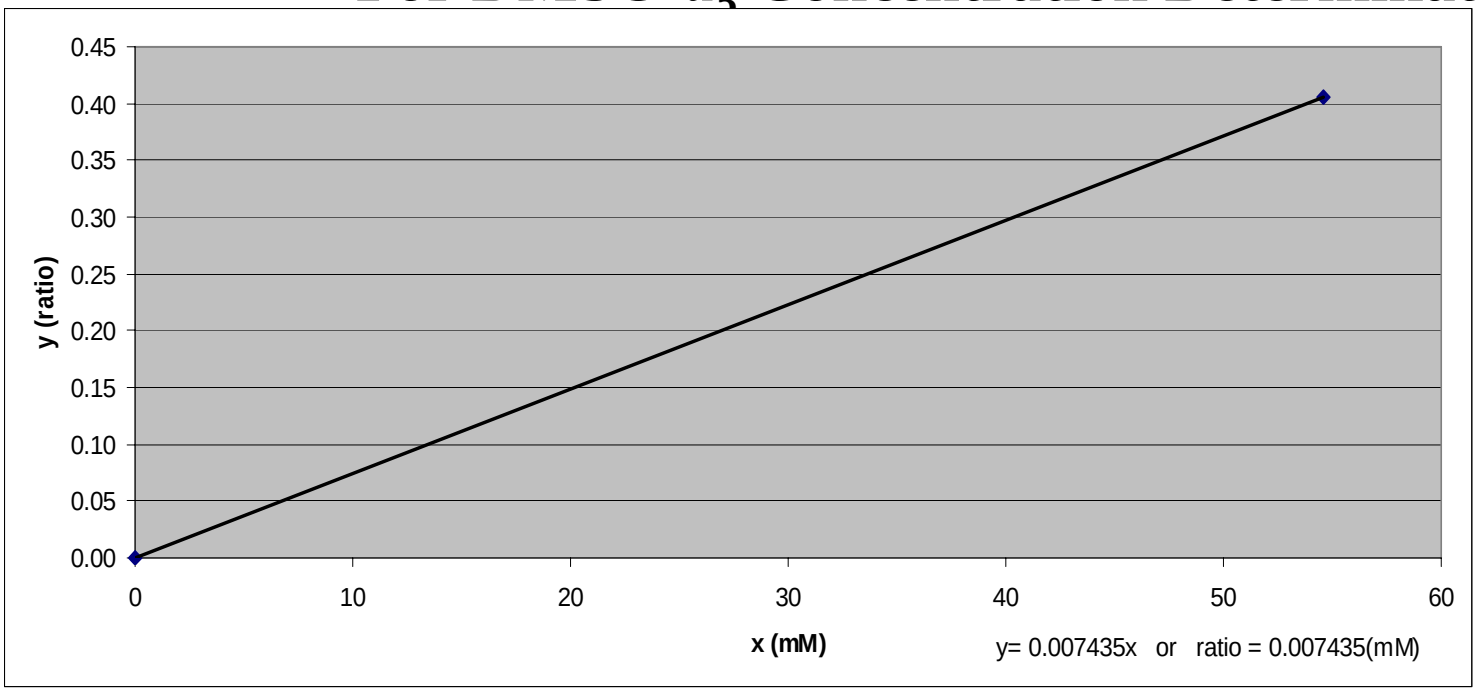
Step 1: Inversion recovery null point experiment. Used to determine which peaks can be used for accurate integration and concentration determination. NB: DMSO signal is nulled and all other signals are positively phased.

S5 Caffeine / DMSO-d_5 Integration Ratios Used to Construct 9-Point Calibration Curve

Caffeine	H:DMSO-d_5 Ratios				
Concentration (mM)	n=1	n=2	n=3	mean	STDev
0.098	0.0013	0.0011	0.0011	0.0012	1.3021E-04
0.295	0.0027	0.0024	0.0025	0.0025	1.6187E-04
0.589	0.0051	0.0054	0.0055	0.0053	1.7227E-04
0.982	0.0101	0.0091	0.0101	0.0098	5.9454E-04
2.945	0.0297	0.0295	0.0294	0.0295	1.5822E-04
5.890	0.0587	0.0586	0.0588	0.0587	9.4197E-05
9.820	0.1026	0.1024	0.1012	0.1021	7.6686E-04
29.450	0.2971	0.2972	0.2970	0.2971	1.2446E-04

- Each sample was measured 3 times (n = 1-3) and H:DMSO-d_5 result averaged and saved under the “mean”
- The standard deviation for the 3 experiments (n=1-3) was calculated
- DMSO-d_6 (Batch # 7H-266)

S6 Single Point Calibration Curve Construction using Caffeine For DMSO-d_5 Concentration Determination



DMSO-d_6 (Batch # 6K-382)

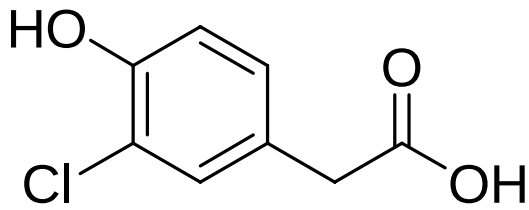
y = H:DMSO-d_5 ratio

x = caffeine concentration (mM)

Worked example for DMSO-d_5 concentration determination:

1. Make up caffeine standard (54.6 mM) in DMSO-d_6 and then run 2-step qNMR experiment
2. Measure ratio of integrals, H:DMSO-d_5 = 0.406
3. Gradient of calibration curve = 0.406/54.6 = 0.007435
4. Concentration of DMSO-d_5 = 1/0.007435 = 134.5 mM

S7 ^{1}H NMR Spectrum of 3-Chloro-4-Hydroxyphenylacetic Acid (sample 11) in DMSO-d_6 Showing Integrals and Calculations for Concentration Determination

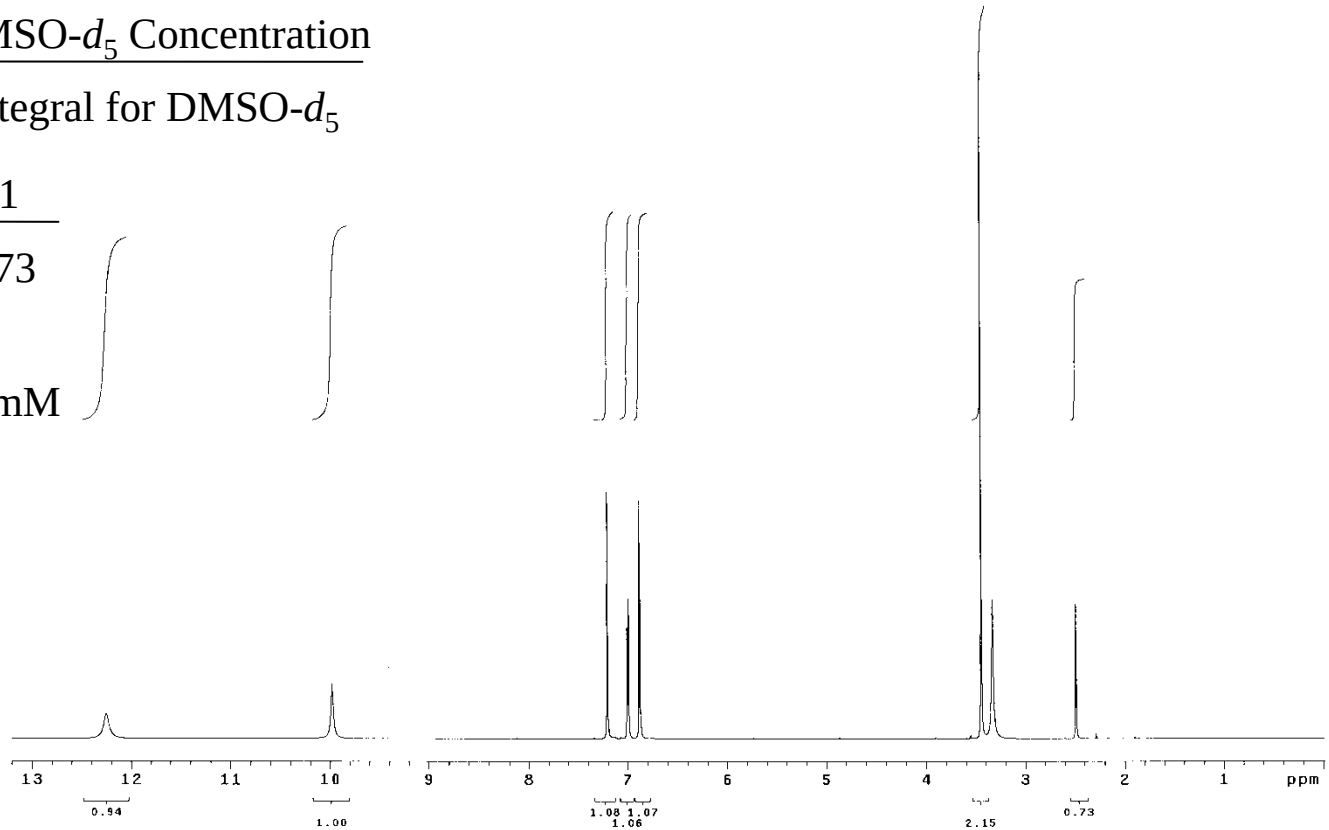


Integral for DMSO–d_5 = 0.73
Integral for 1H of Compound = 7.28 / 7H = 1.04
DMSO-d_5 Concentration (Batch # 6L-488) = 59.1 mM

$$\frac{\text{Compound Concentration}}{\text{Integral for 1H of Compound}} = \frac{\text{DMSO-}d_5\text{ Concentration}}{\text{Integral for DMSO-}d_5}$$

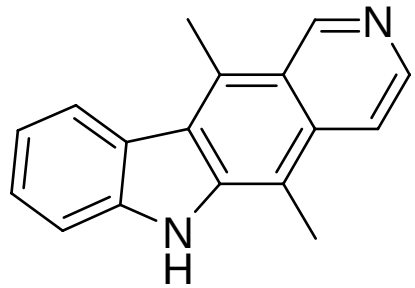
$$\frac{\text{Compound Concentration}}{1.04} = \frac{59.1}{0.73}$$

Compound Concentration = 84.26 mM



S8 ^{1}H NMR Spectrum of Ellipticine (sample 12) in DMSO-d_6 Showing Integrals and Calculations for Concentration Determination

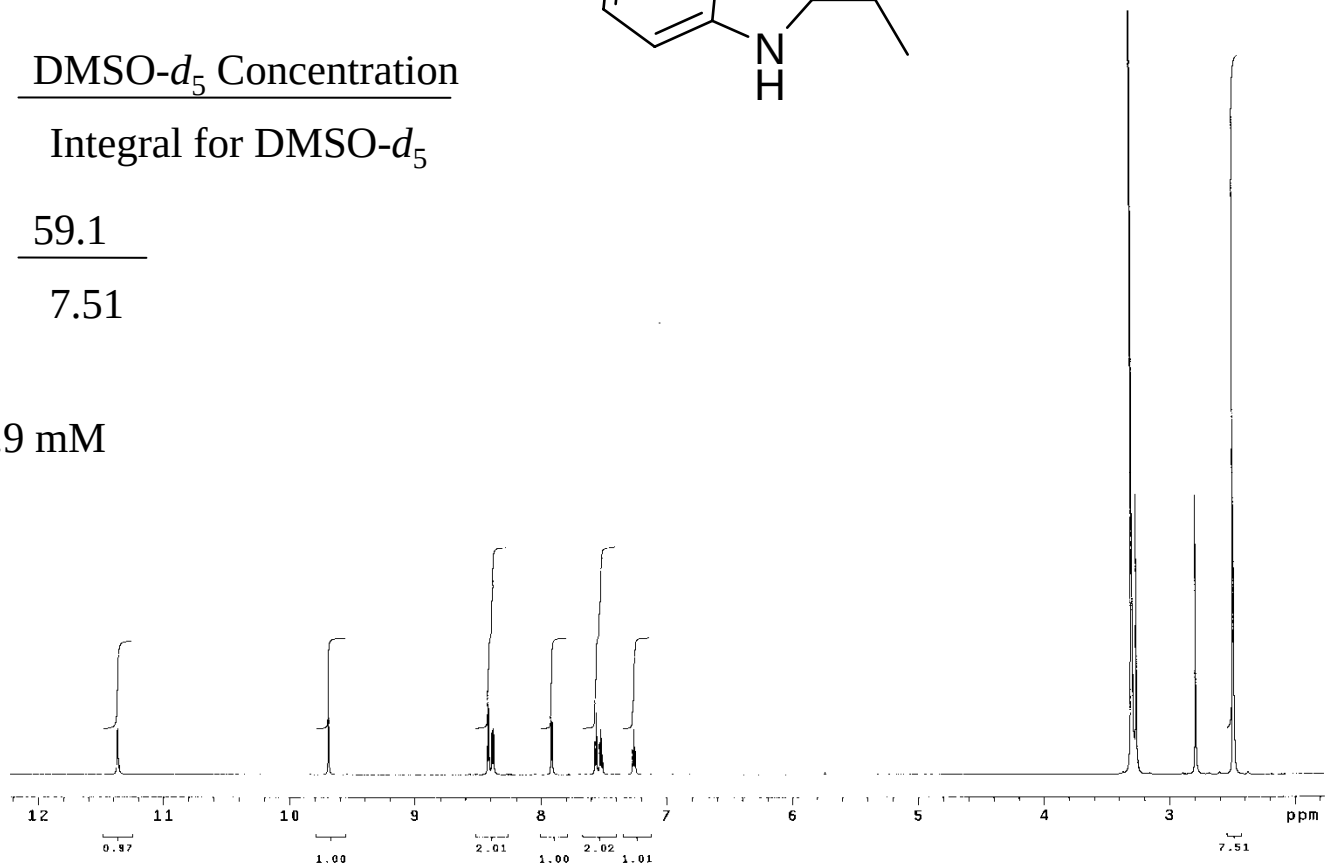
Integral for DMSO–d_5 = 7.51
Integral for 1H of Compound = 8.01 / 8H = 1.001
DMSO-d_5 Concentration (Batch # 6L-488) = 59.1 mM



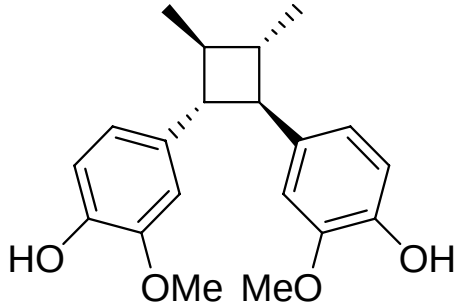
$$\frac{\text{Compound Concentration}}{\text{Integral for 1H of Compound}} = \frac{\text{DMSO-}d_5\text{ Concentration}}{\text{Integral for DMSO-}d_5}$$

$$\frac{\text{Compound Concentration}}{1.001} = \frac{59.1}{7.51}$$

Compound Concentration = 7.9 mM



S9 ^{1}H NMR Spectrum of Endiandrin A (sample 15) in DMSO-d_6 Showing Integrals and Calculations for Concentration Determination

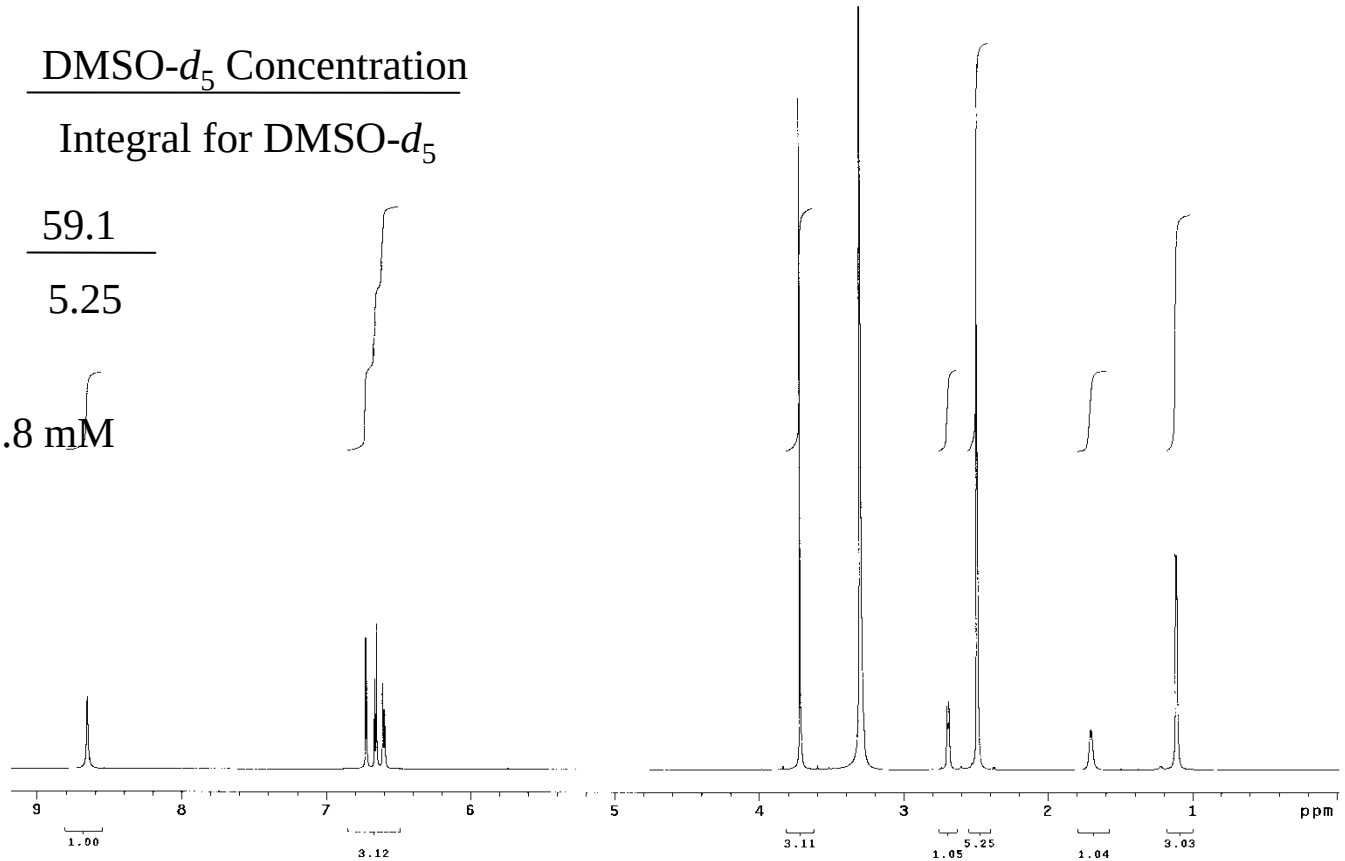


Integral for DMSO–d_5 = 5.25
Integral for 1H of Compound = 12.35 / 24H = 0.5145
DMSO-d_5 Concentration (Batch # 6L-488) = 59.1 mM

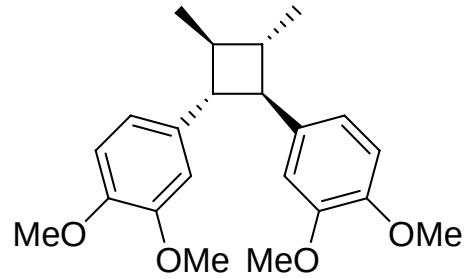
$$\frac{\text{Compound Concentration}}{\text{Integral for 1H of Compound}} = \frac{\text{DMSO-}d_5\text{ Concentration}}{\text{Integral for DMSO-}d_5}$$

$$\frac{\text{Compound Concentration}}{0.5145} = \frac{59.1}{5.25}$$

Compound Concentration = 5.8 mM



S10 ^{1}H NMR Spectrum of Dimethyl-O-endiandrin A (sample 18) in DMSO-d_6 Showing Integrals and Calculations for Concentration Determination



Integral for DMSO–d_5 = 4.24
Integral for 1H of Compound = 14.27 / 28H = 0.509
DMSO-d_5 Concentration (Batch # 6L-488) = 59.1 mM

$$\frac{\text{Compound Concentration}}{\text{Integral for 1H of Compound}} = \frac{\text{DMSO-}d_5\text{ Concentration}}{\text{Integral for DMSO-}d_5}$$

$$\frac{\text{Compound Concentration}}{0.509} = \frac{59.1}{4.24}$$

Compound Concentration = 7.1 mM