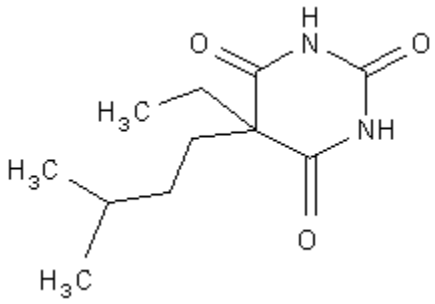
AMOBARBITAL

Latest Revision: January 23, 2006



1. SYNONYMS

CFR: Amobarbital

CAS #: Free acid: 57-43-2
Sodium salt: 64-43-7

Other names: 5-Ethyl-5-(3-methylbutyl)-2,4,6-pyrimidinetrione
5-Ethyl-5-isopentylbarbituric acid
Amytal

2. CHEMICAL AND PHYSICAL DATA

2.1. CHEMICAL DATA

Form	Chemical Formula	Molecular Weight	Melting Point (°C)
Free acid	$C_{11}H_{18}N_2O_3$	226.2	155-158
Sodium salt	$C_{11}H_{17}N_2NaO_3$	248.2	156

2.2. SOLUBILITY

Form	A	C	E	H	M	W
Free acid	***	S	FS	***	FS	VSS
Sodium salt	***	I	I	***	FS	VS

A = acetone, C = chloroform, E = ether, H = hexane, M = methanol and W = water, VS = very soluble, FS = freely soluble, S = soluble, PS = sparingly soluble, SS = slightly soluble, VSS = very slightly soluble and I = insoluble

Note: A saturated aqueous solution of the free acid has a pH about 5.6. A 10% aqueous solution of the sodium salt has a pH not more than 11.

3. SCREENING TECHNIQUES

3.1. COLOR TESTS

REAGENT	COLOR PRODUCED
Dille-Koppanyi	Violet
Zwikker's	Violet
Mercurous nitrate	Black

3.2. CRYSTAL TESTS

REAGENT	CRYSTALS FORMED
Wagenaar's	Light blue needles in clusters
Acetic acid	Long, branching needles and hexagonal plates

3.3. THIN-LAYER CHROMATOGRAPHY

Visualization

Mercurous nitrate spray Acidified potassium permanganate

COMPOUND	RELATIVE R_1	
	System TLC7	System TLC12
barbituric acid	0.0	0.0
phenobarbital	0.9	0.5
amobarbital	**1.0**	**1.0**
pentobarbital	1.0	1.0
secobarbital	1.0	1.0
thiobarbital	1.4	1.0

3.4. GAS CHROMATOGRAPHY

All gas chromatographic methods should be performed on the free acid of the barbiturate only, due to the poor chromatography of the sodium salts. The run time may be shortened by using an isothermal run if no late eluting components are present in the sample.

Method AMO-GCS1

Instrument: Gas chromatograph operated in split mode with FID

Column: 5% phenyl/95% methyl silicone 12 m x 0.2 mm x 0.33 µm film thickness

Carrier gas: Helium at 0.5 mL/min

Temperatures: Injector: 230°C
Detector: 250°C
Oven program:
1) 175°C initial temperature for 6.0 min
2) Ramp to 260°C at 30°C/min
3) Hold final temperature for 10.0 min

Injection Parameters: Split Ratio = 50:1, 1 µL injected

For the free acid, samples are to be dissolved in chloroform and filtered. For the sodium salt, samples are initially added to 0.5 N sulfuric acid followed by extraction into chloroform prior to filtering and injection.

COMPOUND	RRT	COMPOUND	RRT
amphetamine	0.21	**amobarbital**	**1.00 (4.08 min)**
pentadecane	0.45	secobarbital	1.31
ephedrine	0.48	caffeine	1.52
butabarbital	0.83	methaqualone	2.22
acetaminophen	0.87	codeine	2.58

3.5. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Method AMO-LCS1

Instrument: High performance liquid chromatograph equipped with diode array

Column: 5 µm ODS, 150 mm x 3.2 mm

Detector: UV, 220 nm

Flow: 0.750 mL/min

Injection Volume: 10.0 µL

Mobile Phase: 59% H_2O, 1% glacial acetic acid, 40% methanol, 0.02 M methanesulfonic acid, adjust pH to 3.5 with 0.1 N sodium hydroxide

Samples are to be dissolved in methanol and filtered with a 0.45-micron filter.

COMPOUND	RRT	COMPOUND	RRT
amphetamine	does not elute	butabarbital	0.51
acetaminophen	0.30	cocaine	0.73
codeine	0.33	quinine	0.81
caffeine	0.42	**amobarbital**	**1.00 (5.08 min)**
aspirin	0.43	pentobarbital	1.00
heroin	0.51	secobarbital	1.25
phenobarbital	0.51	methaqualone	1.85

4. SEPARATION TECHNIQUES

The free acid may be extracted using dry chloroform or ether, if no other similarly soluble compounds are present. Alternatively, solvent extraction is performed by first dissolving the sample in an alkaline solution and extracting with chloroform or ether, and discarding the chloroform or ether layer. The solution is then acidified and the free acid is extracted with chloroform. Evaporation of the chloroform results in a white powder.

The sodium salt can be isolated by first placing the sample in 0.5 N sulfuric acid. After shaking, the converted free acid can be extracted into chloroform.

5. QUANTITATIVE PROCEDURES

5.1. GAS CHROMATOGRAPHY

All gas chromatographic methods used to quantitate barbiturates should be run on the free acids only, due to the poor chromatography of the sodium salts.

Method AMO-GCQ1

Internal Standard Stock Solution:
0.4 mg/mL docosane in chloroform.

Standard Solution Preparation:
Accurately weigh and prepare a standard solution of amobarbital (free acid) at approximately 0.6 mg/mL using above internal standard stock solution.

Sample Preparation:
Accurately weigh an amount of sample into a volumetric flask and dilute with internal standard stock solution. If necessary, dilute the sample so the final concentration approximates the standard concentration.

Instrument:	Gas chromatograph operated in split mode with FID
Column:	5% phenyl/95% methyl silicone 12.5 m x 0.2 mm x 0.33 µm film thickness
Carrier gas:	Helium at 1.1 mL/min
Temperatures:	Injector: 250°C Detector: 260°C Oven program: 210°C isothermal
Injection Parameters:	Split Ratio = 60:1, 1 µL injected
Typical Retention Time:	Amobarbital: 1.08 min Docosane: 3.70 min
Linear Range:	0.1 - 1.0 mg/mL
Repeatability:	RSD less than 1.2%
Correlation Coefficient:	0.999
Accuracy:	Error less than 5%

COMPOUND	RRT	COMPOUND	RRT
dimethylsulfone	0.37	diphenhydramine	1.51
nicotinamide	0.54	lidocaine	1.57
amphetamine	0.55	phenobarbital	1.94
ephedrine	0.56	procaine	2.35

benzocaine	0.68	docosane	3.42
methamphetamine	0.70	methaqualone	3.55
ibuprofen	0.76	cocaine	>5.0
acetaminophen	0.91	tetracaine	>5.0
phenacetin	0.94	tetracosane	>5.0
amobarbital	**1.00 (1.08 min)**	codeine	>5.0
pentobarbital	1.06	morphine	>5.0
secobarbital	1.19	heroin	>5.0
caffeine	1.39	quinine	>5.0

5.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPH

Method AMO-LCQ1

Standard Solution Preparation:
Accurately weigh and prepare a standard solution of amobarbital (free acid or sodium salt) at approximately 0.8 mg/mL using methanol.

Sample Preparation:
Accurately weigh an amount of sample into a volumetric flask and dilute with methanol. If necessary, dilute the sample so the final concentration approximates the standard concentration. Filter sample with 0.45-micron filter.

Instrument:	High performance liquid chromatograph equipped with diode array
Column:	5 µm ODS, 150 mm x 3.2 mm
Detector:	UV, 220 nm
Flow:	0.750 mL/min
Injection Volume:	10.0 µL
Mobile Phase:	59% H_2O, 1% glacial acetic acid, 40% methanol, 0.02 M methanesulfonic acid, adjust pH to 3.5 with 0.1 N sodium hydroxide
Typical Retention Time:	Amobarbital: 5.08 min
Linear Range:	0.4 - 1.6 mg/mL
Repeatability:	RSD less than 1.0%

Correlation Coefficient: 0.999

Accuracy: Error less than 5%

6. QUALITATIVE DATA

6.1. INFRARED SPECTROSCOPY (FT-IR)

An additional difficulty in comparing the IR spectra of amobarbital arises from the existence of different crystalline forms or polymorphs which generate differences in spectra. To overcome this difficulty, both sample and standard should be subjected to the same preparations.

See spectra on the following pages for Mass Spectrometry, FT-IR, Vapor Phase IR, and Nuclear Magnetic Resonance.

7. REFERENCES

Fulton, C.L., *Modern Microcrystal Tests for Drugs,* Wiley - Interscience, 1969.

Horwitz, William, Ed., *Official Methods of Analysis of the Association of Official Analytical Chemists, 12th ed.*, Association of Official Analytical Chemists, 1975.

Clarke, E.G.C., *Isolation and Identification of Drugs, 2nd Edition*, The Pharmaceutical Press, 1986.

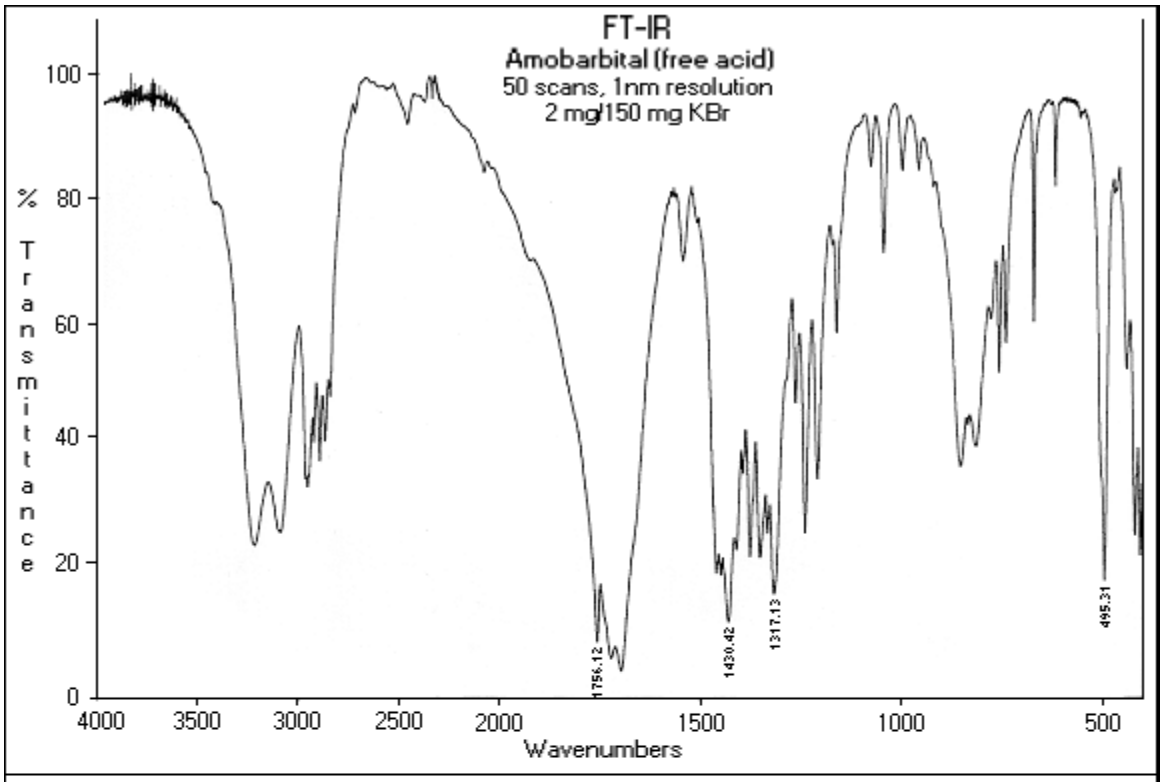
Budavari, S., *The Merck Index, 12th Edition*, Merck and Co., Inc., 1996, p. 95.

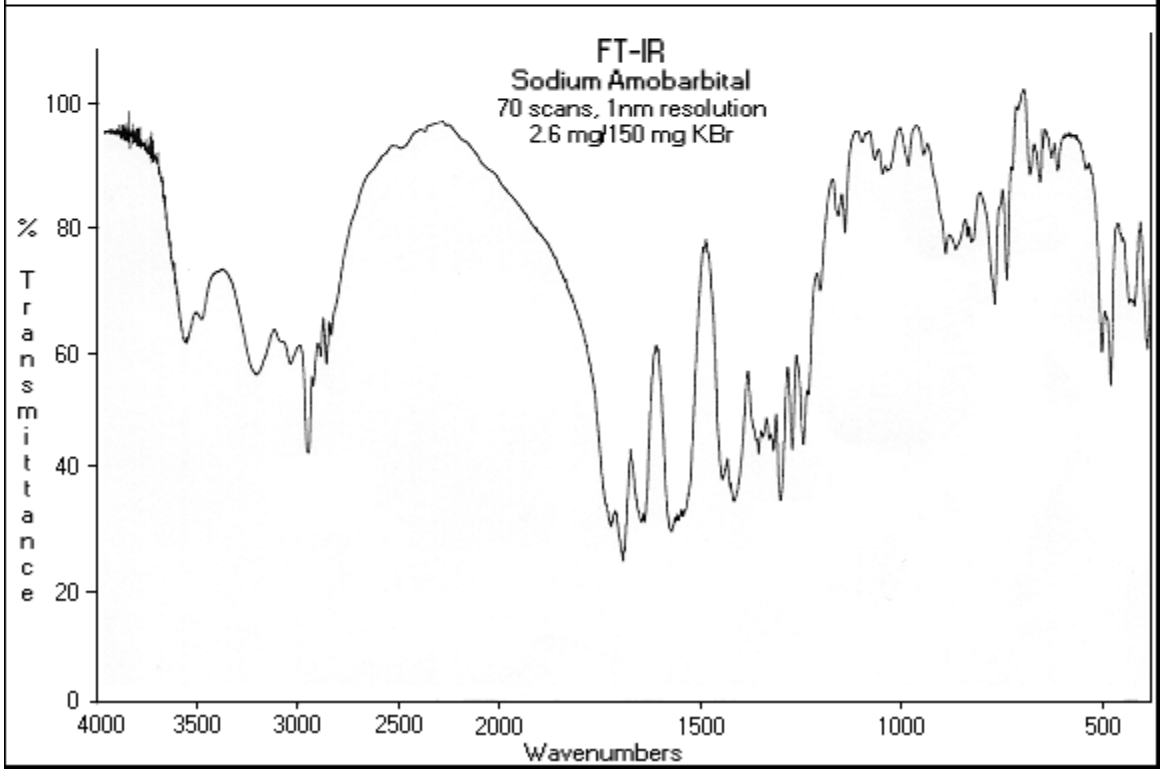
Saferstein, Richard, Ph.D., *Forensic Science Handbook, Volume II*, Prentice Hall, 1988.

8. ADDITIONAL RESOURCES

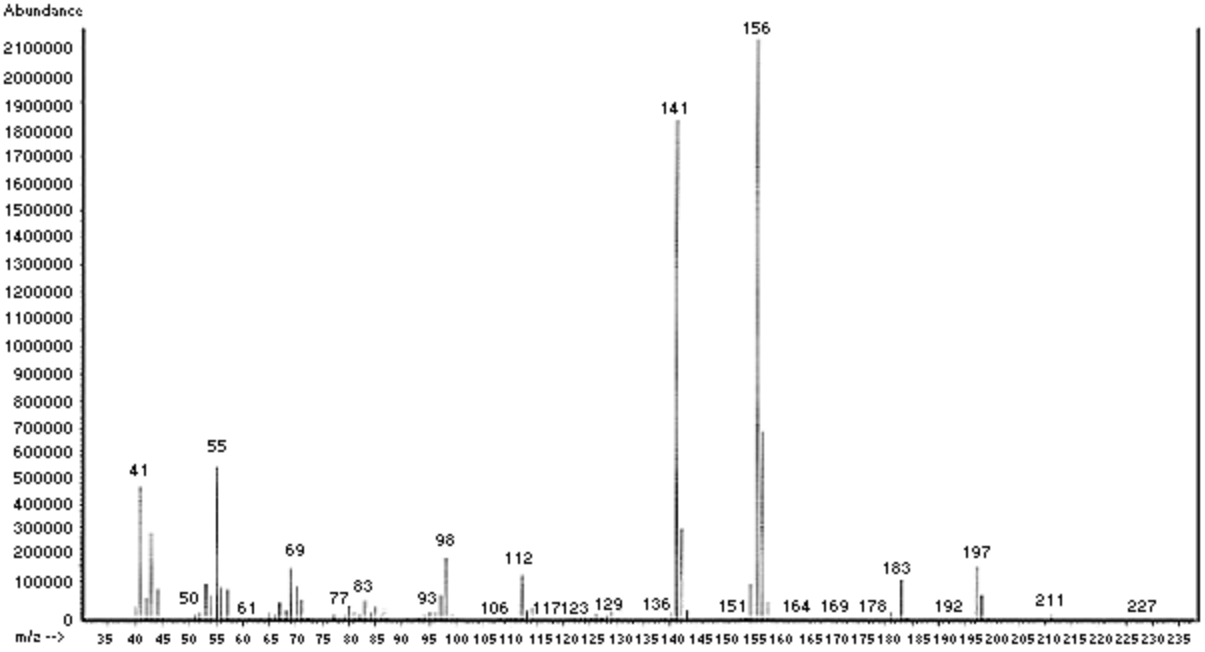
Forendex

Wikipedia


FT-IR
Amobarbital (free acid)
50 scans, 1nm resolution
2 mg/150 mg KBr
% Transmittance
100
80
60
40
20
0
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers
1756.12
1430.42
1317.13
495.31

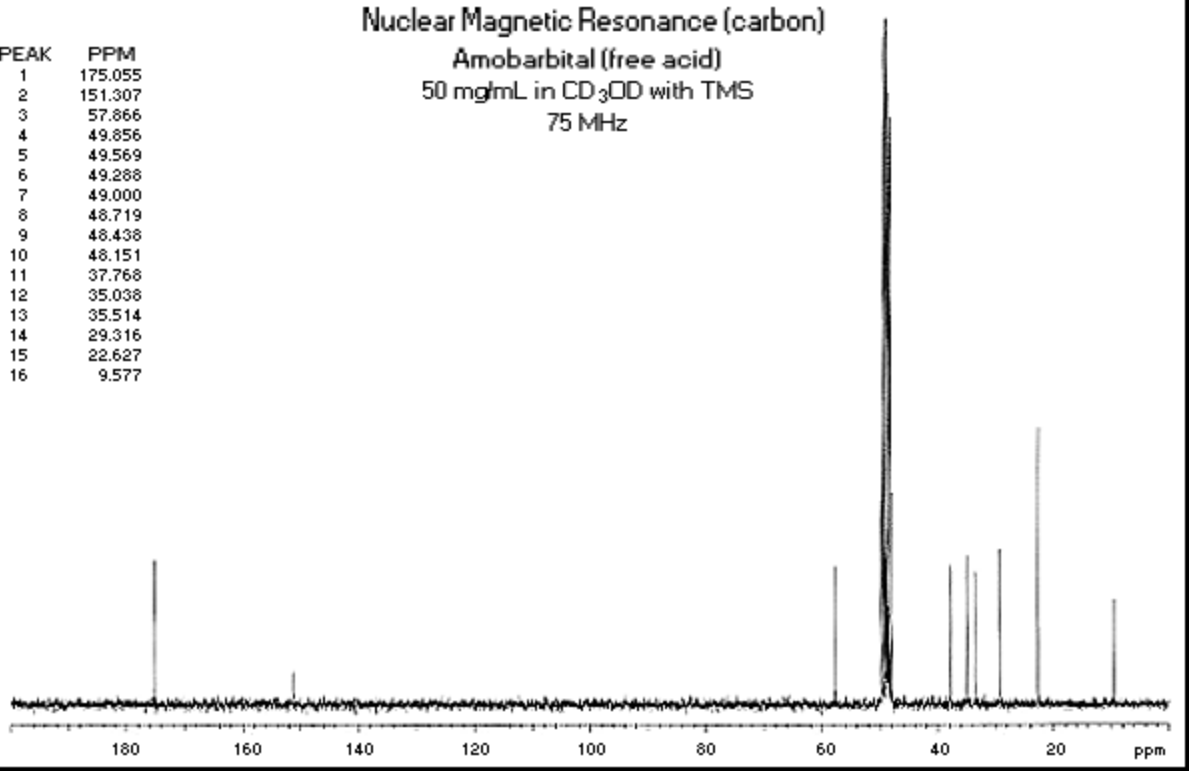

FT-IR
Sodium Amobarbital
70 scans, 1nm resolution
2.6 mg/150 mg KBr
% Transmittance
100
80
60
40
20
0
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers

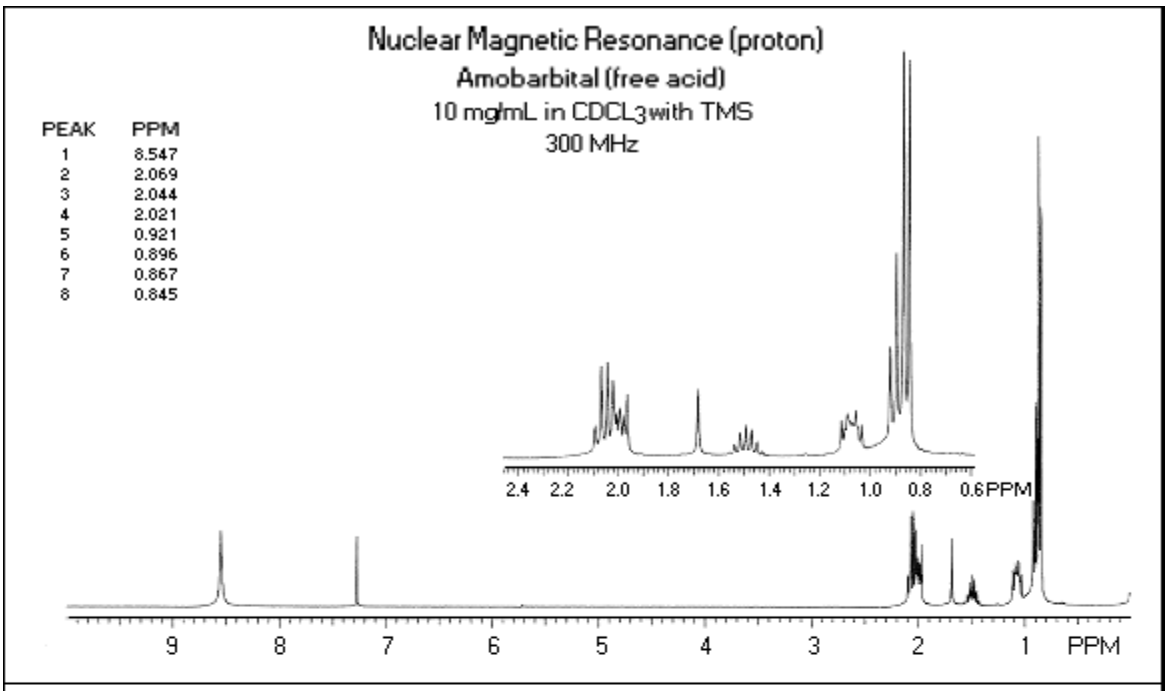
Mass Spectrometry
Amobarbital

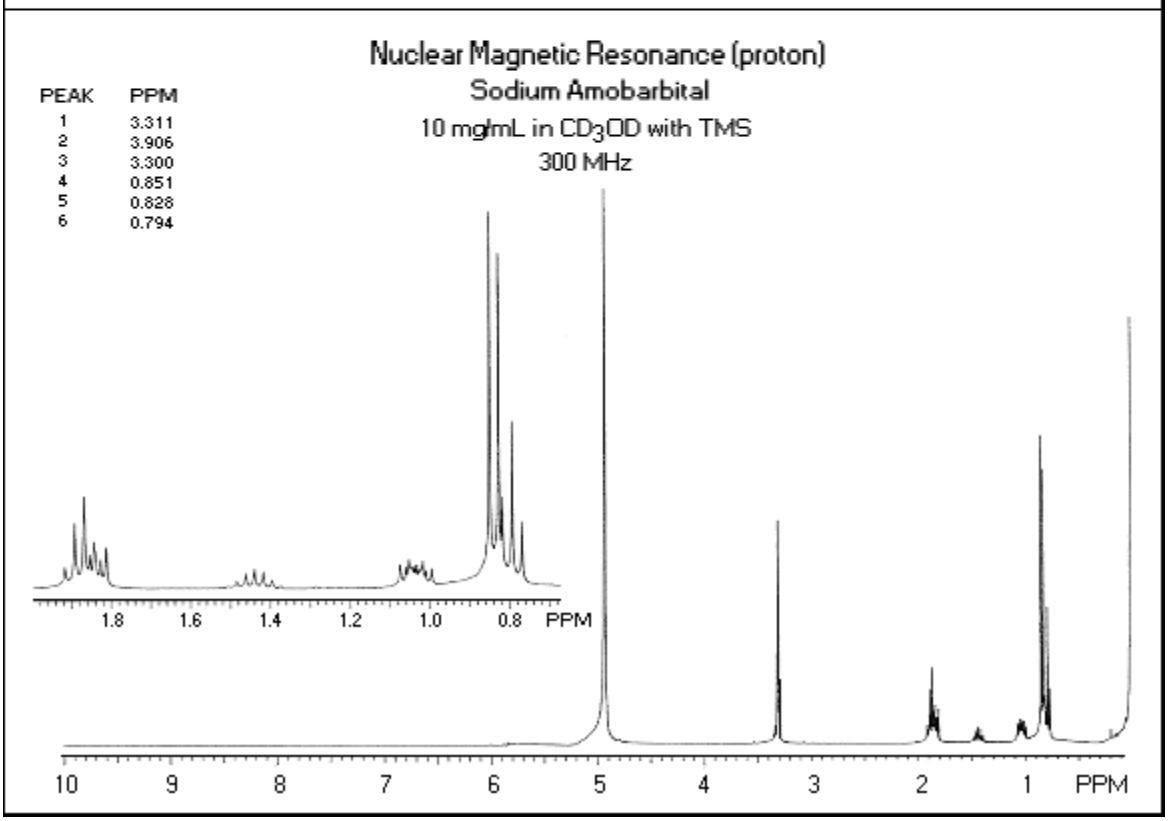


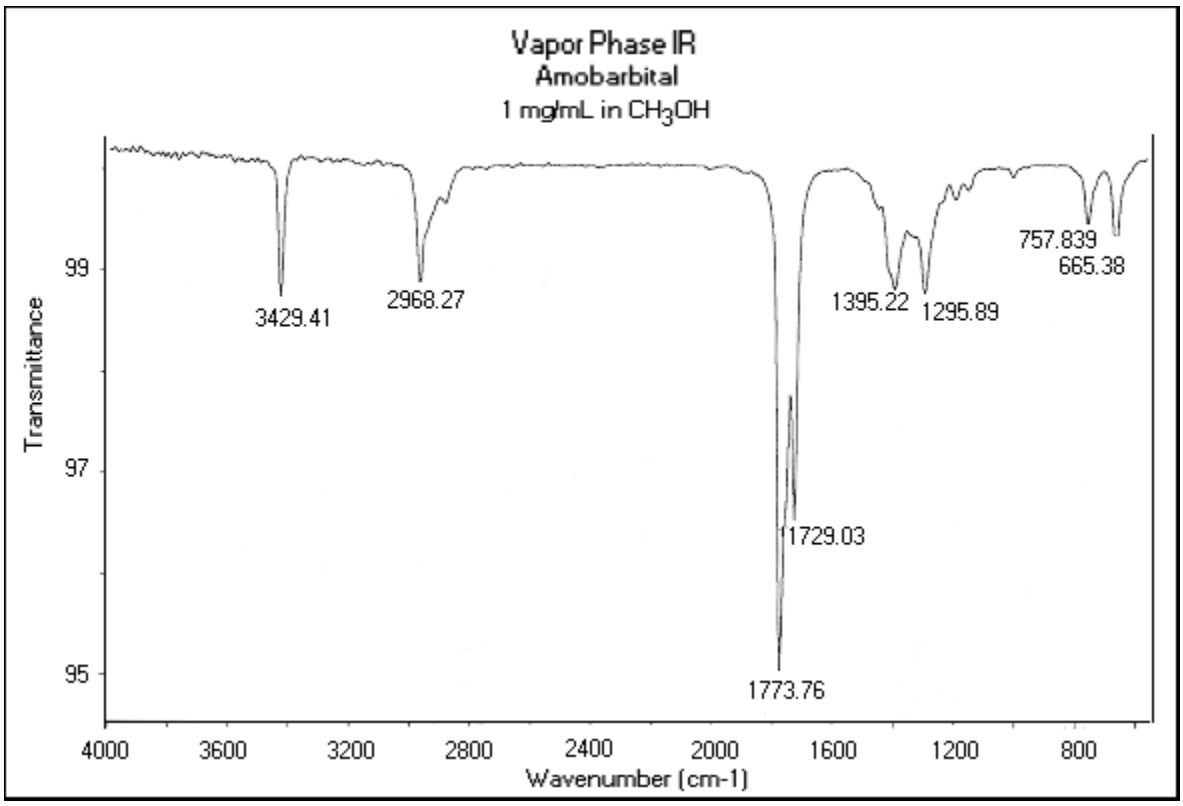
Nuclear Magnetic Resonance (carbon)
Amobarbital (free acid)
50 mg/mL in CD_3OD with TMS
75 MHz

PEAK	PPM
1	175.055
2	151.307
3	57.866
4	49.856
5	49.569
6	49.288
7	49.000
8	48.719
9	48.438
10	48.151
11	37.768
12	35.038
13	35.514
14	29.316
15	22.627
16	9.577




Nuclear Magnetic Resonance (proton)
Amobarbital (free acid)
10 mg/mL in CDCL3 with TMS
300 MHz
PEAK PPM
1 8.547
2 2.069
3 2.044
4 2.021
5 0.921
6 0.896
7 0.867
8 0.845
2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 PPM
9 8 7 6 5 4 3 2 1 PPM


Nuclear Magnetic Resonance (proton)
Sodium Amobarbital
10 mg/mL in CD3OD with TMS
300 MHz
PEAK PPM
1 3.311
2 3.906
3 3.300
4 0.851
5 0.828
6 0.794
1.8 1.6 1.4 1.2 1.0 0.8 PPM
10 9 8 7 6 5 4 3 2 1 PPM



***No data available