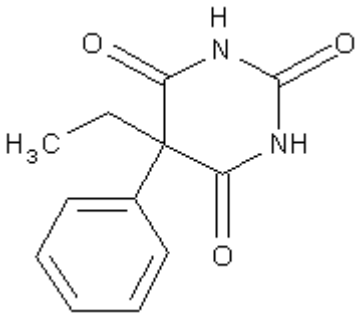
PHENOBARBITAL

Latest Revision: February 15, 1999



1. SYNONYMS

CFR: Phenobarbital

CAS #: Free acid: 50-06-6
Sodium salt: 57-30-7

Other Names: Fenobarbital
Phenemalum
Phenobarbitone
Phenylethylbarbituric acid
Phenylethylmalonylurea
5-Ethyl-5-phenylbarbituric acid
Phenemalnatrium
Phenobarbitone sodium
Sodium phenylethylbarbiturate

2. CHEMICAL AND PHYSICAL DATA

2.1. CHEMICAL DATA

Form	Chemical Formula	Molecular Weight	Melting Point (°C)
Free acid	$C_{12}H_{12}N_2O_3$	232.2	174-178
Sodium salt	$C_{12}H_{11}N_2NaO_3$	254.2	~175

2.2. SOLUBILITY

Form	A	C	E	H	M	W
Free acid	***	PS	S	***	FS	SS
Sodium salt	***	I	I	***	S	FS

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: Phenobarbital is soluble in alkali hydroxides and carbonates, freely soluble in alcohol. Sodium phenobarbital is soluble in ethanol.

3. SCREENING TECHNIQUES

3.1. COLOR TESTS

REAGENT	COLOR PRODUCED
Dille-Koppanyi	Purple
Zwikker's	Purple in the chloroform layer (very weak blue if one drop glacial acetic acid added)

3.2. CRYSTAL TESTS

REAGENT	CRYSTALS FORMED
Wagenaar's	Small rectangular prisms
Ammoniacal nickel acetate	Single rectangular crystals

3.3. THIN LAYER CHROMATOGRAPHY

Visualization

UV light at 254 nm both before and after exposure to ammonia vapor

COMPOUND	RELATIVE R_1 System TLC7
barbituric acid	0.0

phenobarbital	1.0
amobarbital	1.1
pentobarbital	1.2
secobarbital	1.2
thiobarbital	1.6

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 PHE-GCS1

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 1.0 mL/min

Temperatures: Injector: 270°C
Detector: 280°C
Oven program:
1) 175°C initial temperature for 1.0 min
2) Ramp to 270°C at 15°C/min
3) Hold final temperature for 2.0 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
acetaminophen	0.56	caffeine	0.8
amobarbital	0.62	**phenobarbital**	**1.00 (3.86 min)**
pentobarbital	0.66	tetracosane	1.57
secobarbital	0.73	heroin	1.99

3.5. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Method PHE-LCS

Instrument: High performance liquid chromatograph equipped with diode array

Column: 5µm 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	1.25
acetaminophen	0.6	cocaine	1.47
codeine	0.67	quinine	1.62
caffeine	0.84	amobarbital	2
aspirin	0.84	pentobarbital	2
heroin	1	secobarbital	2.5
phenobarbital	**1.00 (2.55 min)**	methaqualone	3.67

3.6. SALT FORM DETERMINATION

For quantitative purposes, it is necessary to know whether the barbiturate is present as a free acid or in a salt form. The solubility test is generally applicable to bulk powder. As excipients in tablets and capsules may interfere with the observation, the pH test may be more appropriate for those preparations.

Solubility:
Place small amounts of the suspect material in two test tubes. Add several drops of water to the first test tube and several drops of ethyl acetate to the second. Observe in which solvent the material dissolves. Free acids are soluble in organic solvents such as ethyl acetate, but are insoluble in water. The salt forms of phenobarbital

are readily soluble in water, but are insoluble in ethyl acetate. Other organic solvents such as ether and chloroform may be substituted for ethyl acetate.

pH Determination:
Place 10 to 20 mg of phenobarbital in a test tube and add 10 mL of water. Determine the pH. A pH greater than 8.0, indicates that the phenobarbital is present as the sodium or calcium salt.

4. SEPARATION TECHNIQUES

Virtually all of the barbiturates encountered in illicit traffic appear in the form of tablets, capsules and bulk powder diverted from legitimate sources. They are present as the sodium or calcium salt or the free acid. These salt forms 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.

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.

5. QUANTITATIVE PROCEDURES

5.1. GAS CHROMATOGRAPHY

Method PHE-GCQ1
Internal Standard Stock Solution:
0.4 mg/mL docosane in chloroform.

Standard Solution Preparation:
Accurately weigh and prepare a standard solution of phenobarbital (free acid) at approximately 0.5 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 1.0 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: Phenobarbital: 2.10 min
Docosane: 3.70 min

Linear Range: 0.1 - 1.0 mg/mL

Repeatability: RSD less than 1.0%

Correlation Coefficient: Base: 0.999

Accuracy: Error less than 5%

COMPOUND	RRT	COMPOUND	RRT
dimethylsulfone	0.19	diphenhydramine	0.65
nicotinamide	0.23	lidocaine	0.68
amphetamine	0.24	**phenobarbital**	**1.00 (2.10 min)**
ephedrine	0.24	procaine	1.21
benzocaine	0.29	docosane	1.76
methamphetamine	0.3	methaqualone	1.83
ibuprofen	0.33	cocaine	>2.5
acetaminophen	0.39	tetracaine	>2.5
phenacetin	0.41	tetracosane	>2.5
amobarbital	0.43	codeine	>2.5
pentobarbital	0.46	morphine	>2.5
secobarbital	0.52	heroin	>2.5
caffeine	0.6	quinine	>2.5

5.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Method PHE-LCQ1

Internal Standard Stock Solution:
0.1 mg/mL strychnine in buffer: acetonitrile 50:50.

Standard Solution Preparation:
Accurately weigh and prepare a standard solution of phenobarbital (free acid or sodium salt) at approximately 0.5 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. 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, 210 nm
Flow:	1.5 mL/min
Injection Volume:	5.0 µL
Buffer:	4000 mL distilled water, 10 g sodium hydroxide, 30.0 mL phosphoric acid and 8.0 mL hexylamine
Mobile Phase:	Buffer: acetonitrile 75:25
Typical Retention Time:	Phenobarbital: 4.37 min Strychnine: 1.16 min
Linear Range:	0.1 - 1.0 mg/mL
Repeatability:	RSD less than 1.0%
Correlation Coefficient:	0.999
Accuracy:	Error less than 5%

COMPOUND	RRT	COMPOUND	RRT
strychnine	0.28	amobarbital	1.99
heroin	0.36	pentobarbital	1.99
phenobarbital	**1.00 (4.37 min)**	secobarbital	2.77

6. QUALITATIVE DATA

6.1. ULTRAVIOLET SPECTROPHOTOMETRY

SOLVENT	MAXIMUM ABSORBANCE (NM)
0.05 M sodium tetraborate	239

6.2. INFRARED SPECTROSCOPY (FT-IR)

An additional difficulty in comparing the IR spectra of phenobarbital 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 FT-IR, Mass Spectrometry, Nuclear Magnetic Resonance, and Vapor Phase IR.

7. REFERENCES

Billups N.F., The American Drug Index, Philadelphia, 1996.

Budavari, S., The Merck Index, 12th Edition, Merck and Co., Inc., 1996, p. 1246.

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

Methods of Analysis, Internal Revenue Service Publication 341, revised June 1967.

Mills T. and Robertson J.C, Instrumental Data for Drug Analysis, New York, 1987.

Physician's Desk Reference, 38th Edition, Medical Economics Company (Oradell, N.J.), 1998.

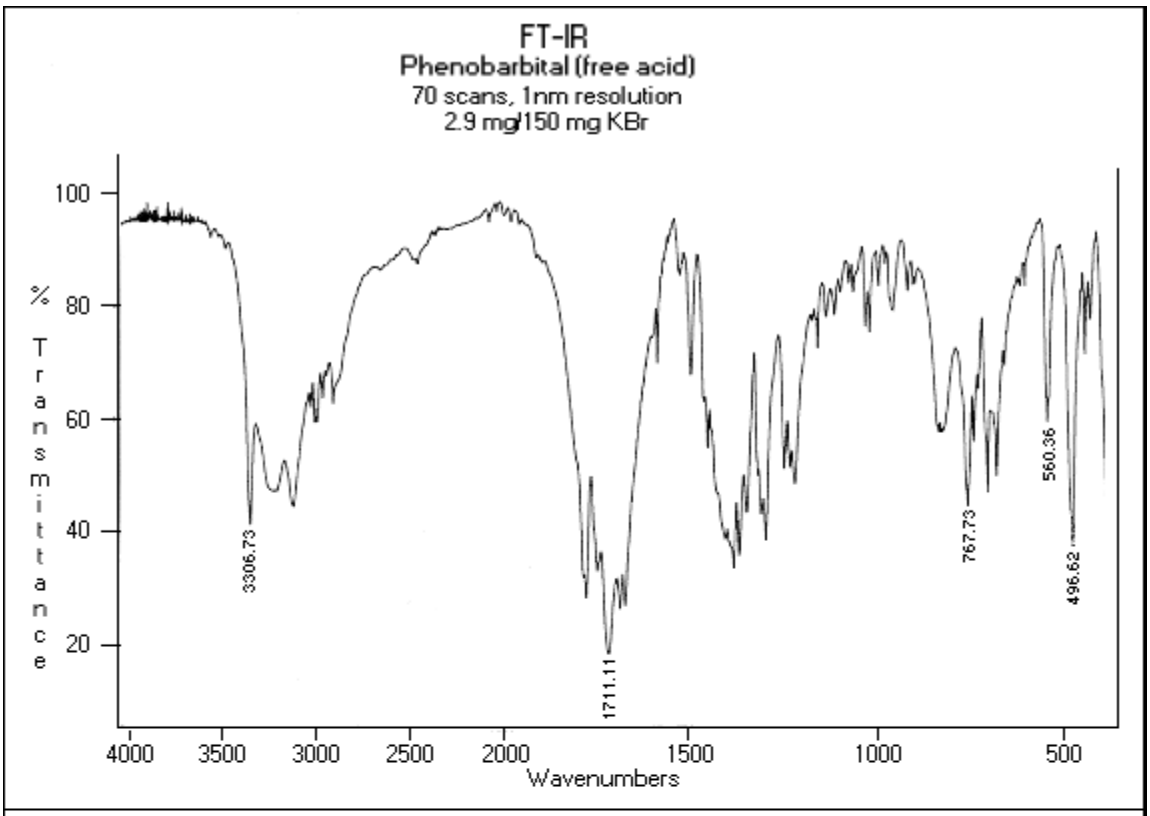
Rapid Testing Methods of Drugs of Abuse, ST/NAR/13, United Nations, New York, 1988.

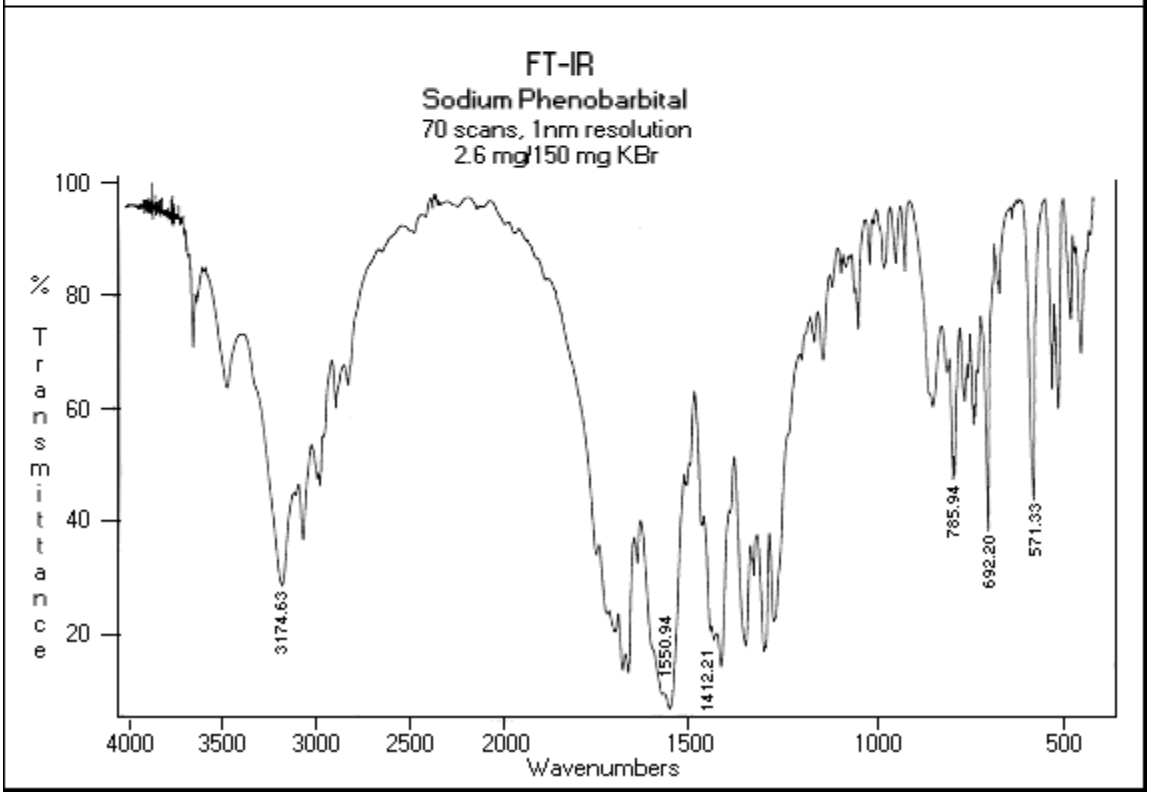
The Logo Index for Tablets and Capsules, U.S. Department of Justice, Drug Enforcement Administration, Washington, D.C., 1996.

8. ADDITIONAL RESOURCES

Forendex

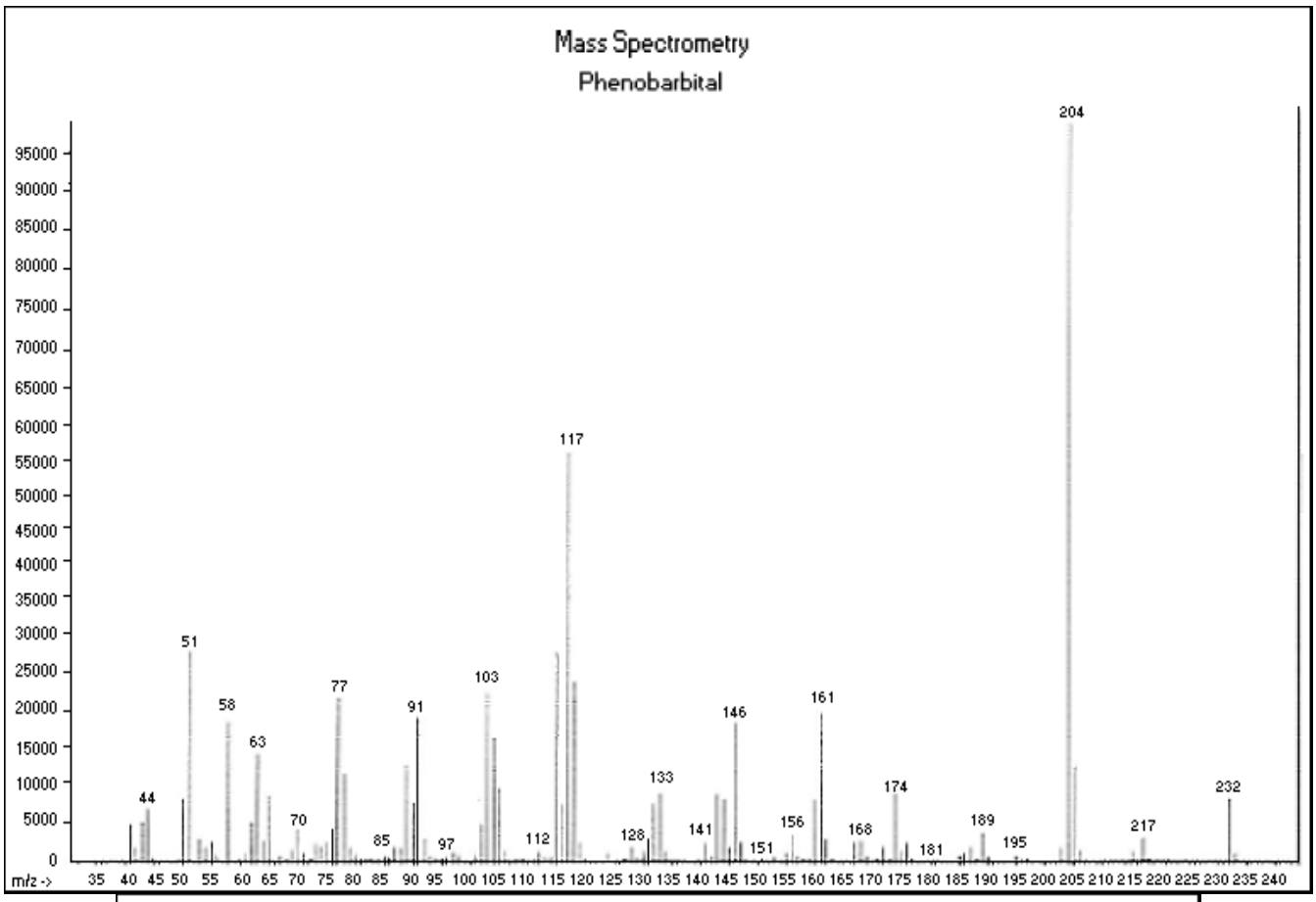
Wikipedia


FT-IR
Phenobarbital (free acid)
70 scans, 1nm resolution
2.9 mg/150 mg KBr
% Transmittance
100
80
60
40
20
3306.73
1711.11
767.73
560.36
496.62
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers


FT-IR
Sodium Phenobarbital
70 scans, 1nm resolution
2.6 mg/150 mg KBr
% Transmittance
100
80
60
40
20
3174.63
1550.94
1412.21
785.94
692.20
571.33
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers

Mass Spectrometry

Phenobarbital



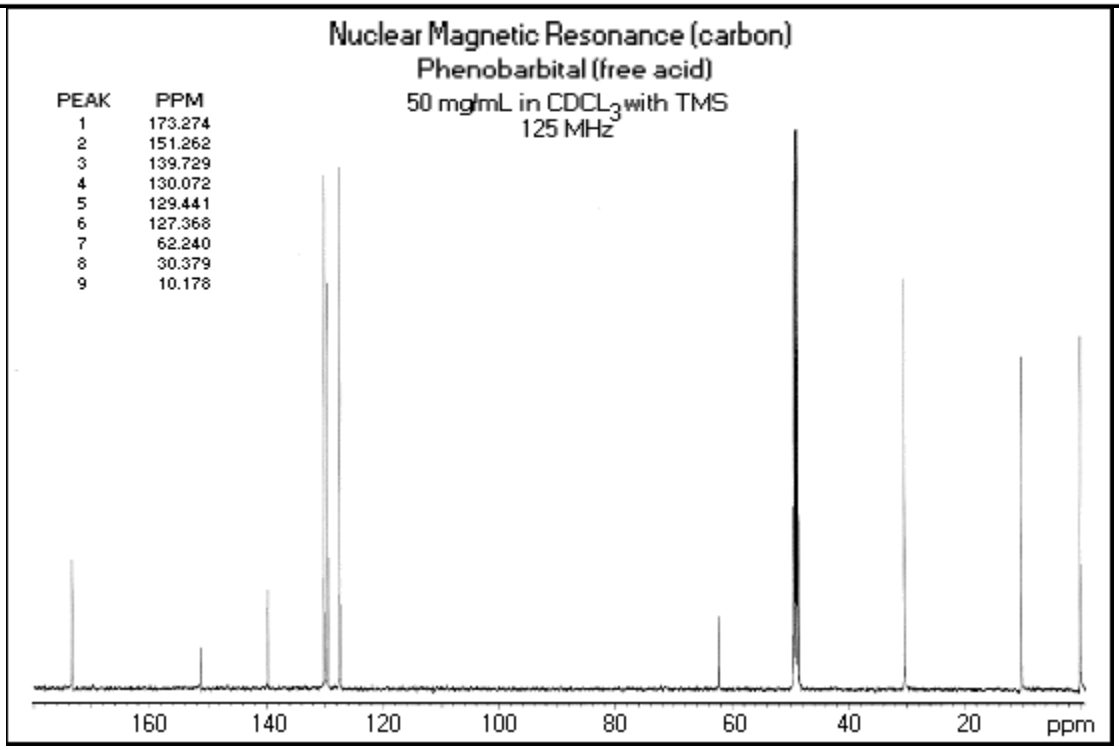
Nuclear Magnetic Resonance (carbon)

Phenobarbital (free acid)

50 mg/mL in $CDCL_3$ with TMS

125 MHz

PEAK	PPM
1	173.274
2	151.262
3	139.729
4	130.072
5	129.441
6	127.368
7	62.240
8	30.379
9	10.178



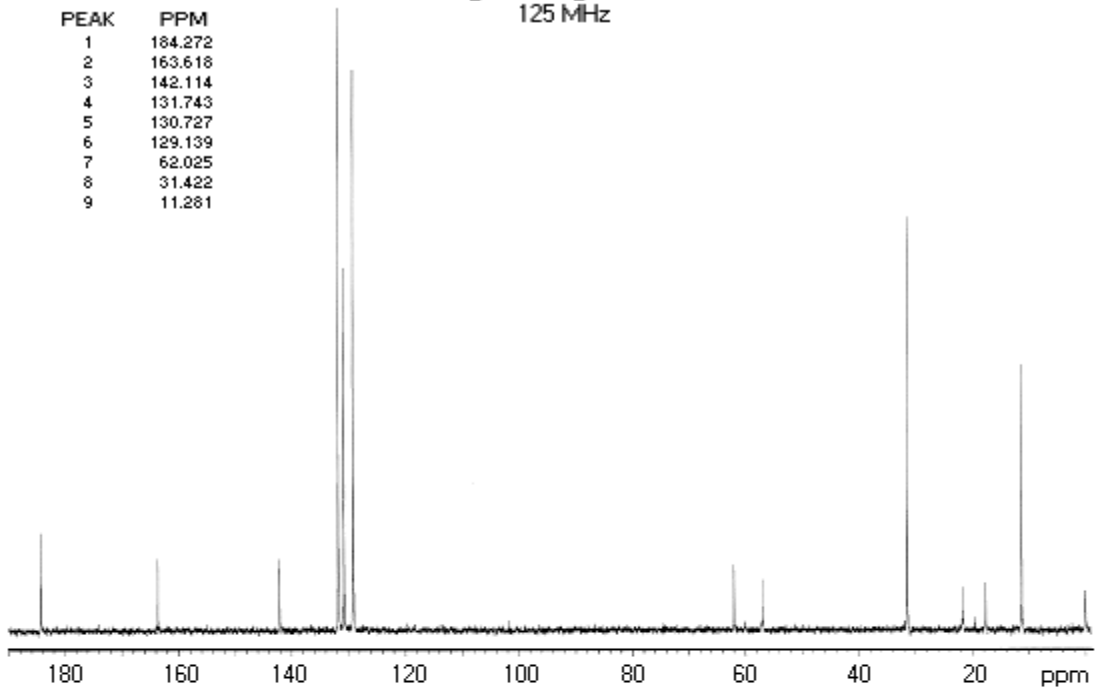
Nuclear Magnetic Resonance (carbon)

Sodium Phenobarbital

50 mg/mL in D_2O with DSS

125 MHz

PEAK	PPM
1	184.272
2	163.618
3	142.114
4	131.743
5	130.727
6	129.139
7	62.025
8	31.422
9	11.281



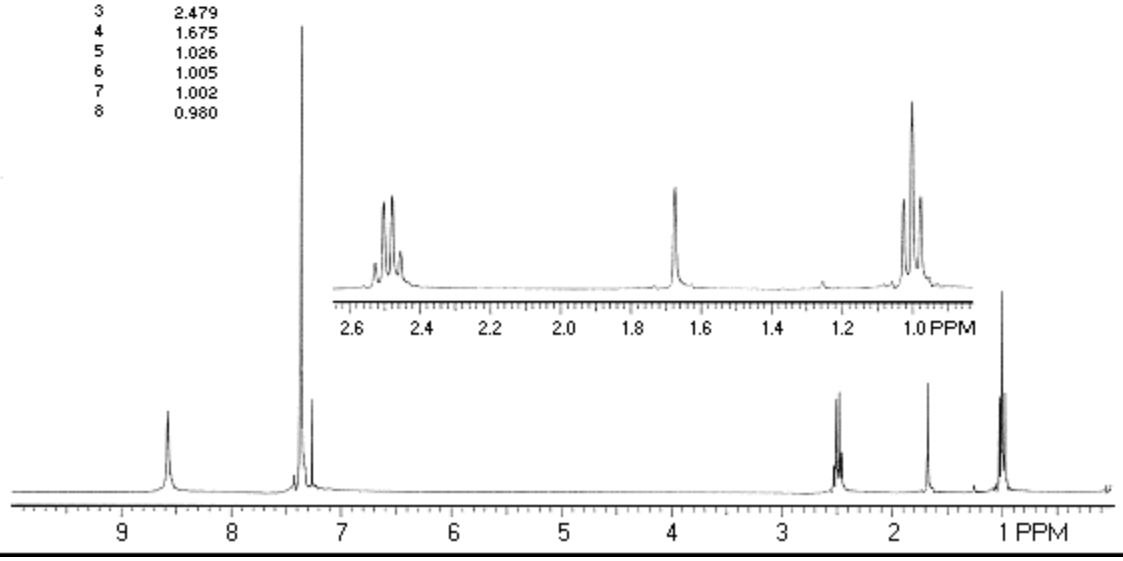
Nuclear Magnetic Resonance (proton)

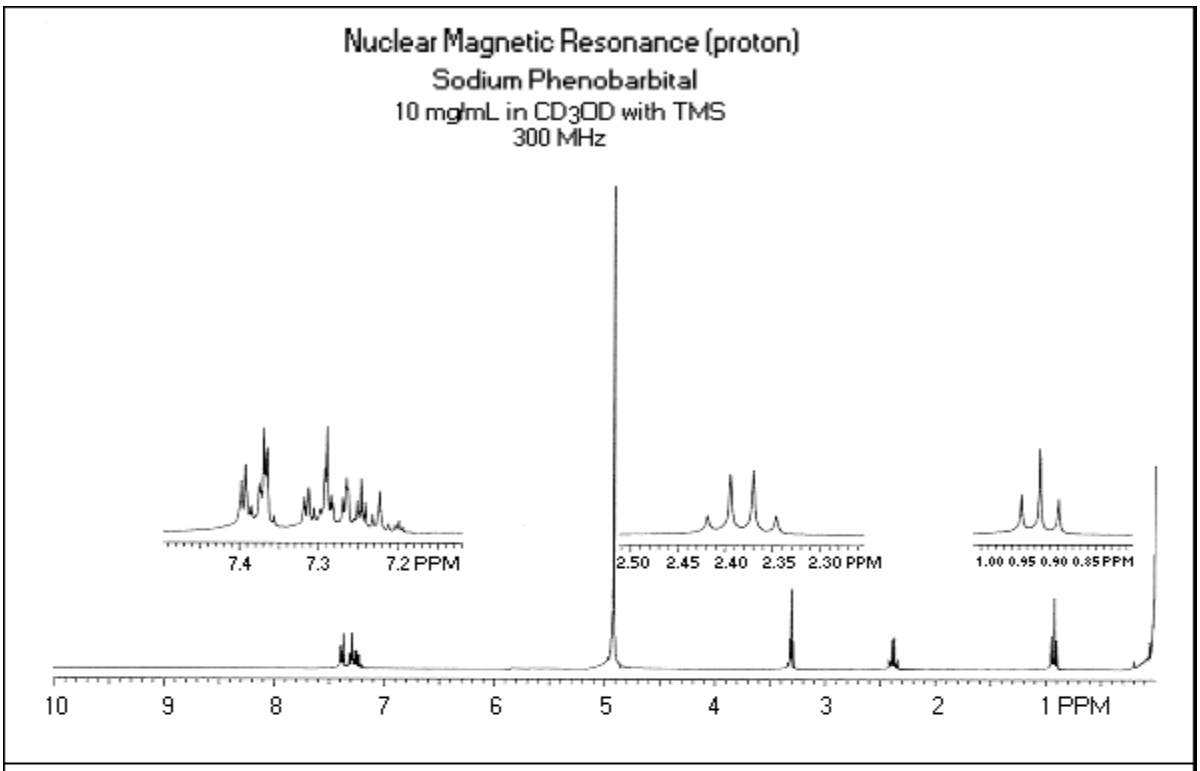
Phenobarbital (free acid)

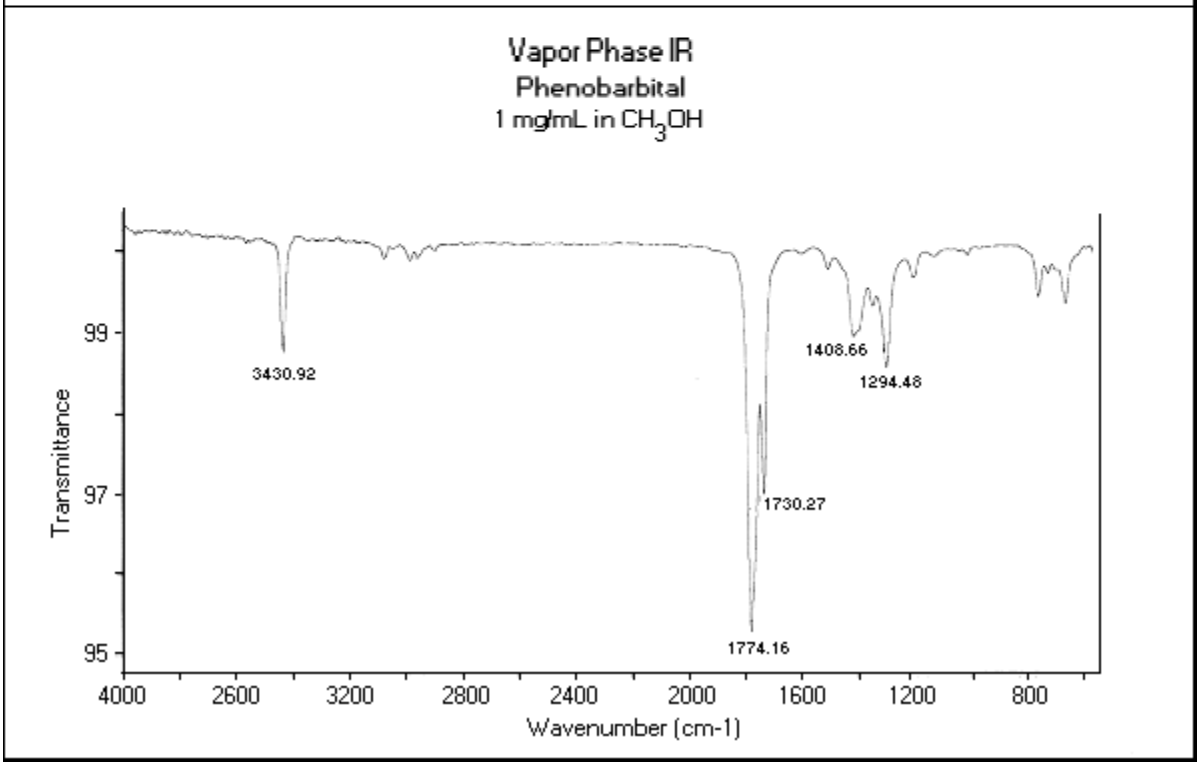
10 mg/mL in $CDCL_3$ with TMS

300 MHz

PEAK	PPM
1	7.363
2	2.503
3	2.479
4	1.675
5	1.026
6	1.005
7	1.002
8	0.980




Nuclear Magnetic Resonance (proton)
Sodium Phenobarbital
10 mg/mL in CD3OD with TMS
300 MHz
7.4
7.3
7.2 PPM
2.50 2.45 2.40 2.35 2.30 PPM
1.00 0.95 0.90 0.85 PPM
10
9
8
7
6
5
4
3
2
1 PPM


Vapor Phase IR
Phenobarbital
1 mg/mL in CH3OH
Transmittance
99
97
95
3430.92
1408.66
1294.48
1730.27
1774.16
4000
2600
3200
2800
2400
2000
1600
1200
800
Wavenumber (cm-1)