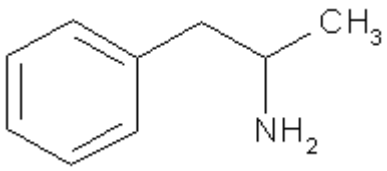
AMPHETAMINE

Latest Revision: May 5, 2005



1. SYNONYMS

CFR: Amphetamine

CAS #: Base: 300-62-9
Hydrochloride: 405-41-4
Sulfate: 60-13-9
Phosphate: 139-10-6

Other Names: α-Methylbenzeneethanamine
α-Methylphenethylamine
1-Phenyl-2-aminopropane
β-Phenylisopropylamine
β-Aminopropylbenzene
Desoxynorephedrine
Phenedrine

2. CHEMICAL AND PHYSICAL DATA

2.1. CHEMICAL DATA

Form	Chemical Formula	Molecular Weight	Melting/Boiling Point (°C)
Base	$C_9H_{13}N$	135.2	BP: 200-203
Hydrochloride	$C_9H_{14}NCl$	171.6	***
Sulfate	$C_{18}H_{28}N_2SO_4$	368.5	MP: Decomposes over 300°C
Phosphate	$C_9H_{13}N \cdot H_3PO_4$	233.2	MP: Sinters at 150°C Decomposes ~300°C

2.2. SOLUBILITY

Form	A	C	E	H	M	W
Base	S	VS	VS	S	S	PS

Hydrochloride	PS	S	I	I	S	S
Sulfate	I	I	I	I	S	FS
Phosphate	***	I	I	***	SS	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

*** No data available .

3. SCREENING TECHNIQUES

3.1. COLOR TESTS

REAGENT	COLOR PRODUCED
Marquis	Orange to brown
Mandelin's	Green, darkens rapidly
Liebermann's	Red/orange

3.2. CRYSTAL TESTS

REAGENT	CRYSTALS FORMED
Gold chloride	Thin, flat, feathery, leaf shaped crystals, low birefringence; some X's and thin birefringent rods
Platinic chloride	Needles, narrow irregular blades of low birefringence
Gold bromide	Trapezoidal blades or small red cigars

Note: Hanging drop technique effective for some mixtures.

3.3. THIN-LAYER CHROMATOGRAPHY

Visualization

1% Ninhydrin in methanol (heat at 100^{o}C 2-3 min)

COMPOUND	RELATIVE R_1 System TLC8
amphetamine	1.0
methamphetamine	0.8
ephedrine	0.9

3.4. GAS CHROMATOGRAPHY

Method AMP-GCS1

Internal Standard Stock Solution:
0.05 mg/mL tetracosane in chloroform:methanol (4:1).

Standard Solution Preparation:
Accurately weigh and prepare standard solutions at approximately 0.05 mg/mL using above internal standard stock solution.

Sample Preparation:
Weigh approximately 20 mg into a GC vial (~2 mL). Fill with internal standard stock solution. If necessary, filter sample through glass wool.

Instrument:	Agilent 6890 Series (or equivalent) 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 1.0 mL/min for 5 min ramped to 2.0 mL/min.
Temperatures:	Injector: 270°C Detector: 280°C Oven program: 1) 175°C initial temperature for 1.0 min 2) Ramp to 280°C at 15°C/min 3) Hold final temperature for 4.0 min
Injection Parameters:	Split Ratio = 60:1, 1 µL injection
Typical Retention Time:	Amphetamine: 0.77 min Tetracosane: 6.01 min

COMPOUND	RRT	COMPOUND	RRT
dimethyl sulfone	0.77	methadone HCl	6.78
amphetamine sulfate	**1.00**	propoxyphene HCl	7.01
methamphetamine	1.08	atropine sulfate	7.05
N,N-dimethyl-amphetamine	1.21	cocaine HCl	7.09
phenylpropanolamine HCl	1.44	tetracaine HCl	7.17
niacinamide	1.64	triprolidine	7.39
methylephedrine	1.78	tetracosane	7.79
MDA HCl	2.08	phenylbutazone	8.02
MDMA HCl	2.34	codeine phosphate	8.10
benzocaine	2.57	morphine sulfate	8.39
MDEA	2.60	diazepam	8.43
guaifenesin	3.10	hydrocodone bitartrate	8.48
acetaminophen	3.23	acetylcodeine	8.85
phenacetin	3.37	monoacetylmorphine	8.94
caffeine	4.48	oxycodone base	8.94
ketamine HCl	4.72	benzoylecgonine tartrate	9.26
diphenhydramine HCl	4.74	chloroquine phosphate	9.33
antipyrine	4.83	heroin HCl	9.57
lidocaine HCl	4.86	quinine base	10.51
doxylamine succinate	5.14	quinine HCl	10.51
Aminopyrine	5.17	quinidine HCl	10.52
phenobarbital	5.47	zolpidem	10.55
xylazine	5.61	papaverine	10.71
levamisole	5.62	clonazepam	10.87
dipyrone	5.79	hydroxyzine	10.92
procaine HCl	6.00	alprazolam	11.56
clenbuterol HCl	6.22	diltiazem	11.64
brompheniramine	6.60	noscapine	13.74

3.5. CAPILLARY ELECTROPHORESIS

Method AMP-CES1

Internal Standard Stock Solution:
0.15 mg/mL phenethylamine in 100 mM sodium phosphate buffer at pH of 3.5.

Standard Solution Preparation:
Accurately weigh and prepare a standard solution of d-methamphetamine hydrochloride, l-methamphetamine hydrochloride, d-amphetamine hydrochloride, l-amphetamine hydrochloride, d-ephedrine hydrochloride, l-ephedrine hydrochloride, d-pseudoephedrine hydrochloride and l-pseudoephedrine hydrochloride at

approximately 0.15 mg/mL each 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. Dilute the sample so the final concentration approximates the standard concentration. If necessary, filter the sample with a 0.45 µm filter prior to injection.

Mode:	Free zone
Column:	47 cm x 50 µm fused silica capillary
Run Buffer:	200 mM sodium phosphate buffer with 30 mM hydroxy-propyl-β-cyclodextrin, pH 3.5
Detector:	UV, 210 nm
Voltage:	26 kV
Temperature:	20°C liquid cooled
Injection:	1 s hydrodynamic
Run Time:	12 min
Rinse Time:	2 min

COMPOUND	RMT	COMPOUND	RMT
phenethylamine	0.58	**d-amphetamine**	**1.00 (9.84 min)**
l-pseudoephedrine	0.91	d-pseudoephedrine	1.02
d-ephedrine	0.95	l-methamphetamine	1.04
l-ephedrine	0.96	d-methamphetamine	1.07
l-amphetamine	**0.98**		

4. SEPARATION TECHNIQUES

Dissolve the sample in water and add 0.1 N sodium hydroxide until basic. Extract the amphetamine base from the aqueous layer with hexane. Filter the hexane extract through a bed of anhydrous sodium sulfate. Bubble HCl gas through the hexane to form the hydrochloride salt.

5. QUANTITATIVE PROCEDURES

***5.1.* GAS CHROMATOGRAPHY**

Method AMP-GCQ1

Internal Standard Stock Solution:
0.5 mg/mL eicosane in chloroform.

Standard Solution Preparation:
Accurately weigh and prepare a standard solution of amphetamine hydrochloride 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:	Agilent 6890 Series (or equivalent) 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 1.0 mL/min
Temperatures:	Injector: 280°C Detector: 280°C Oven program: 1) 175°C initial temperature for 2.0 min 2) Ramp to 250°C at 30°C/min

3) Hold final temperature for 1.0 min

Injection Parameters: Split Ratio = 60:1, 1 µL injected

Typical Retention Time: Amphetamine: 1.60 min
Eicosane: 4.81 min

Linear Range: 0.1 - 1.0 mg/mL
Repeatability: RSD less than 2.0%

Correlation Coefficient: 0.999

Accuracy: Error less than 5%

COMPOUND	RRT	COMPOUND	RRT
dimethylsulfone	0.81	diphenhydramine	2.79
amphetamine	**1.00 (1.60 min)**	lidocaine	2.82
methamphetamine	1.06	eicosane	3.00
nicotinamide	1.39	phenobarbital	3.03
ephedrine	1.39	procaine	3.22
benzocaine	1.77	methaqualone	3.67
ibuprofen	1.90	cocaine	3.82
acetaminophen	1.98	tetracaine	3.87
phenacetin	2.25	tetracosane	4.32
amobarbital	2.32	codeine	4.66
pentobarbital	2.40	morphine	4.94
secobarbital	2.54	heroin	6.45
caffeine	2.69	quinine	8.34

Method AMP-GCQ2

This method derivatizes amphetamine with acetic anhydride and gives excellent reproducibility. The standard and sample must be prepared in the same manner.

Internal Standard Stock Solution:
2.0 mg/mL tetradecane in methylene chloride.

Standard Solution Preparation:
Accurately weigh and prepare a standard solution of amphetamine (hydrochloride or base) at approximately 5.0 mg/mL using methanol. Into a 50 mL volumetric flask, add 5 mL of the standard in methanol and 1 mL of acetic anhydride. Stir the mixture then add 1 mL of triethylamine to neutralize excess acetic anhydride. Add 10 mL of internal standard stock solution then dilute to volume with methylene chloride.

Sample Preparation:
Accurately weigh an amount of sample into a volumetric flask and dilute with methanol to approximately 5.0 mg/mL. Into a 50 mL volumetric flask, add 5 mL of the standard in methanol and 1 mL of acetic anhydride. Stir the mixture then add 1 mL of triethylamine to neutralize excess acetic anhydride. Add 10 mL of internal standard stock solution then dilute to volume with methylene chloride.

Instrument:	Agilent 6890 Series (or equivalent) gas chromatograph operated in split mode with FID
Column:	5% phenyl/95% methyl silicone 30 m x 0.32 mm x 0.25 µm film thickness
Carrier gas:	Helium at 1.0 mL/min
Temperatures:	Injector: 250°C Detector: 300°C Oven program: 160°C isothermal
Injection Parameters:	Split Ratio = 20:1, 1 µL injected
Typical Retention Time:	Amphetamine: 3.40 min Tetradecane: 2.33 min
Linear Range:	0.09 - 1.56 mg/mL
Repeatability:	RSD less than 1.0%
Correlation Coefficient:	0.999
Accuracy:	Error less than 5%

5.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Method AMP-LCQ1

Standard Solution Preparation:
Accurately weigh and prepare a standard solution of amphetamine hydrochloride at approximately 0.5 mg/mL

using 0.1 N HCl.

Sample Preparation:
Accurately weigh an amount of sample into a volumetric flask and dilute with 0.1 N HCl. 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 (Agilent 1100 Series or equivalent).
Column:	5µm Octadecylsilyl (ODS), 150 mm x 3.2 mm at 50°C
Detector:	UV, 210 nm
Flow:	1.00 mL/min
Injection Volume:	5.0 µL
Buffer:	4000 mL distilled water, 10 g sodium hydroxide, 30.0 mL phosphoric acid, 8.0 mL hexylamine and 0.1 g sodium azide
Mobile Phase:	90 % Buffer: 10% Acetonitrile
Typical Retention Time:	Amphetamine: 2.46 min
Linear Range:	0.05 - 1.2 mg/mL
Repeatability:	RSD less than 1.0%
Correlation Coefficient:	0.999
Accuracy:	Error less than 5%

5.3. CAPILLARY ELECTROPHORESIS

Method AMP-CEQ1

Solvents:
Celixir Reagent A (MicroSolv).
Celixir accelerator solution B, pH 2.5 (MicroSolv).

Injection Solvent Preparation:
Accurately weigh 1034 mg of sodium phosphate monobasic into a 100 mL volumetric flask. Dilute to volume with HPLC grade water. Adjust pH to approximately 2.6 using phosphoric acid (add drop wise). Transfer contents into 2000 mL volumetric flask with aid of HPLC grade water. Dilute to volume with HPLC grade water. This final solution contains 3.75 mM phosphate, pH 3.2.

Injection Solvent Preparation (Alternate Method):
Transfer entire contents of 250 mL bottle of DEA injection solvent concentrate (MicroSolv) into 5-L volumetric flask. Dilute to volume with Millipore water or equivalent.

Internal Standard Stock Solution:
1.0 mg/mL N-butylamphetamine in 3.8 mM phosphate buffer at pH of 2.5.

Standard Solution Preparation:
Accurately weigh an appropriate amount of standard amphetamine into a volumetric flask to obtain a final concentration of approximately 0.08 mg/mL. Pipette appropriate amount of internal standard solution to obtain a final concentration of 0.1 mg/mL. Dilute to volume with injection solvent. Filter (0.45 µm) approximately 1.0 mL of solution into a 2.0 mL glass vial removing all air bubbles in the bottom of the vial. Cap the vial with a polypropylene cap.

Sample Preparation:
Accurately weigh an amount of sample into a volumetric flask so the final concentration approximates the standard concentration. Pipette appropriate amount of internal standard solution to obtain a final concentration of 0.1 mg/mL. Dilute to volume with injection solvent. Filter (0.45 µm) approximately 1.0 mL of solution into a 2.0 mL glass vial removing all air bubbles in the bottom of the vial. Cap the vial with a polypropylene cap.

Mode:	Free zone using dynamically coated capillaries
Column:	50 cm x 32.2 µm (23.7 cm to detector) fused silica capillary
Conditioning:	0.1 N NaOH; 1 min H_2O CElixir Reagent A (MicroSolv CE); 2 min CElixir Reagent B, pH 2.5 (MicroSolv CE)
Run Buffer:	CElixir Reagent B, pH 2.5 (MicroSolv CE)
Detector:	UV, 210 nm
Voltage:	10 kV
Temperature:	15°C
Injection:	Sample: 50 mbar x 2 s followed by water at 35 mbar x 1 s
Run Time:	6 min
LinearityRange:	0.00318 mg/mL - 0.1 mg/mL
Repeatability:	RSD<2%
Accuracy:	%E<2.4%

Correlation Coefficient (R^2): 0.9998

COMPOUND	RMT	COMPOUND	RMT
doxylamine	0.881	ketamine	1.108
chlorpheniramine	0.903	phenyltoxolamine	1.119
quinine	0.926	*n*-butylamphetamine	1.152
beta-phenethylamine	0.930	dextromethorphan	1.152
chlorquinine	0.935	cocaine	1.164
nicotinamide	0.963	lidocaine	1.187
amphetamine	**1.00 (4.6 min)**	*cis*-cinnamoylcocaine	1.198
methamphetamine	1.017	*trans*-cinnamoylcocaine	1.221
procaine	1.017	benzocaine	1.440
MDA	1.037	benzoylecgonine	1.935
norpseudoephedrine	1.044	acetaminophen	2.431
MDMA	1.053	caffeine	2.465
norephedrine	1.056	guaifenesin	2.465
pseudoephedrine	1.059	P2P	2.581
tetracaine	1.068	DMSO (neutral marker)	2.765
ephedrine	1.074	aspirin	3.122
phenylephrine	1.096	salicylic acid	5.576
MDEA	1.107		

6. QUALITATIVE DATA

6.1. INFRARED SPECTROSCOPY (FT-IR)

An additional difficulty in comparing the IR spectra of amphetamine arises from the existence of different isomers and of ionic exchange with the matrix. 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, FT-Raman and Vapor Phase IR.

6.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY

Sample Preparation:
Dissolve a small amount of sample into 10mM Ammonium Formate buffer at pH 3.7. Filter sample with 0.45-micron filter if necessary.

Instrument:	High performance liquid chromatograph equipped with diode array and mass spectrometer detector (Agilent 1100 series SL or equivalent).
Column:	ES POLAR RP, 80A, 150 mm x 3.0 mm at 40°C
Detector:	UV, 210 nm and 280 nm MSD, Positive Mode, 150 V Fragmentor
Ionization Mode:	API-ES Drying gas-temp 350°C, flow 13.0 L/min 40 psig Nebulizer Pressure Capillary Voltage 4000V
Flow:	0.50 mL/min
Injection Volume:	2.0 µL
Buffer:	10mM Ammonium Formate, pH 3.7
Mobile Phase:	88 % Buffer: 12% Acetonitrile
Typical Retention Time:	Amphetamine: 5.2 min

7. REFERENCES

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

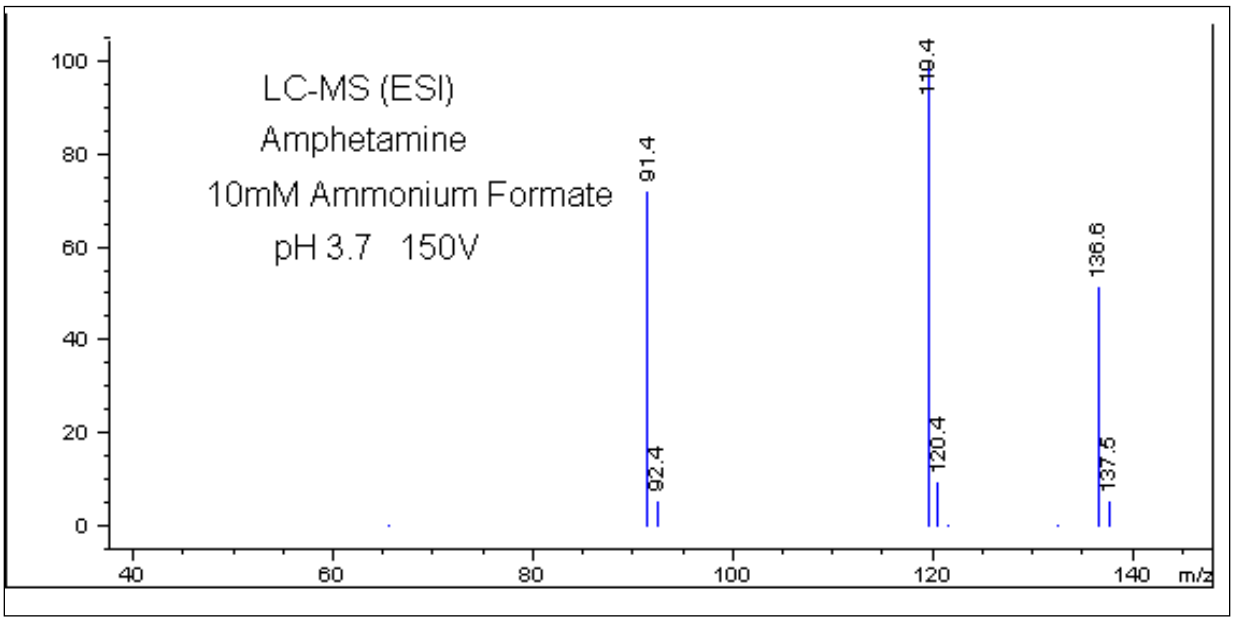
Budavari, S., The Merck Index, 12th Edition, Merck and Co., Inc., 1996, p. 98.

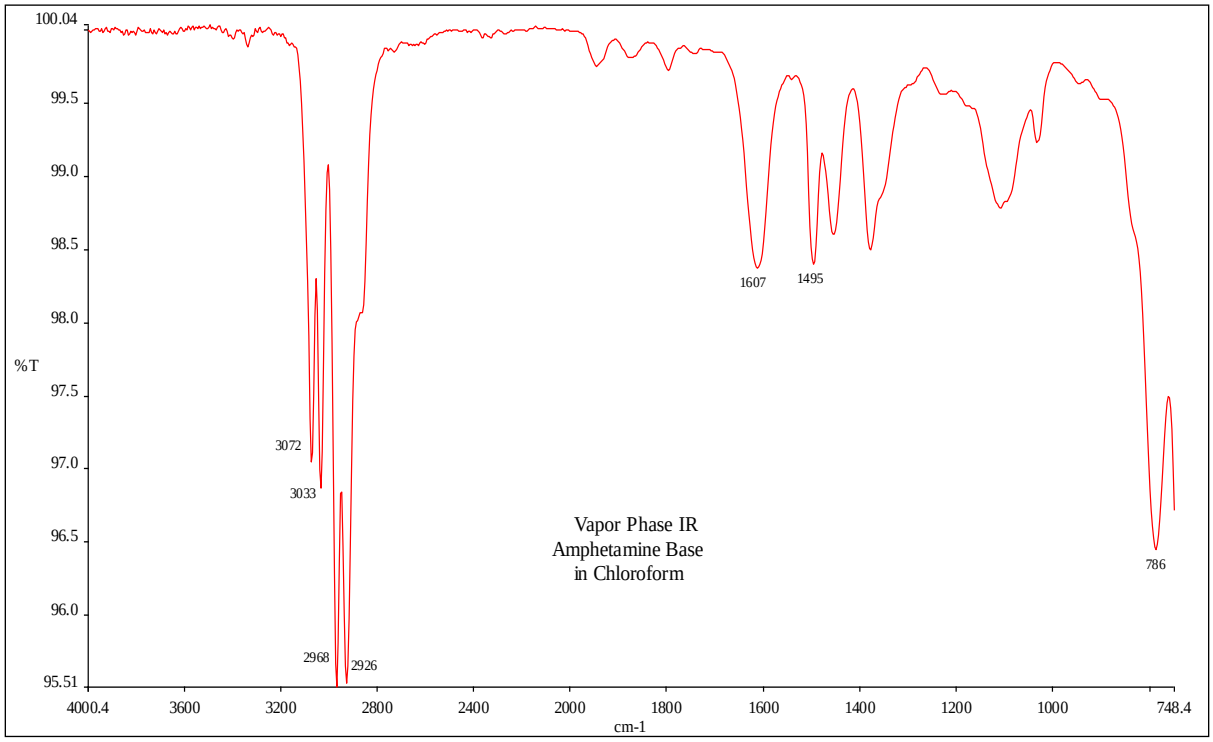
Special Testing and Research Laboratory

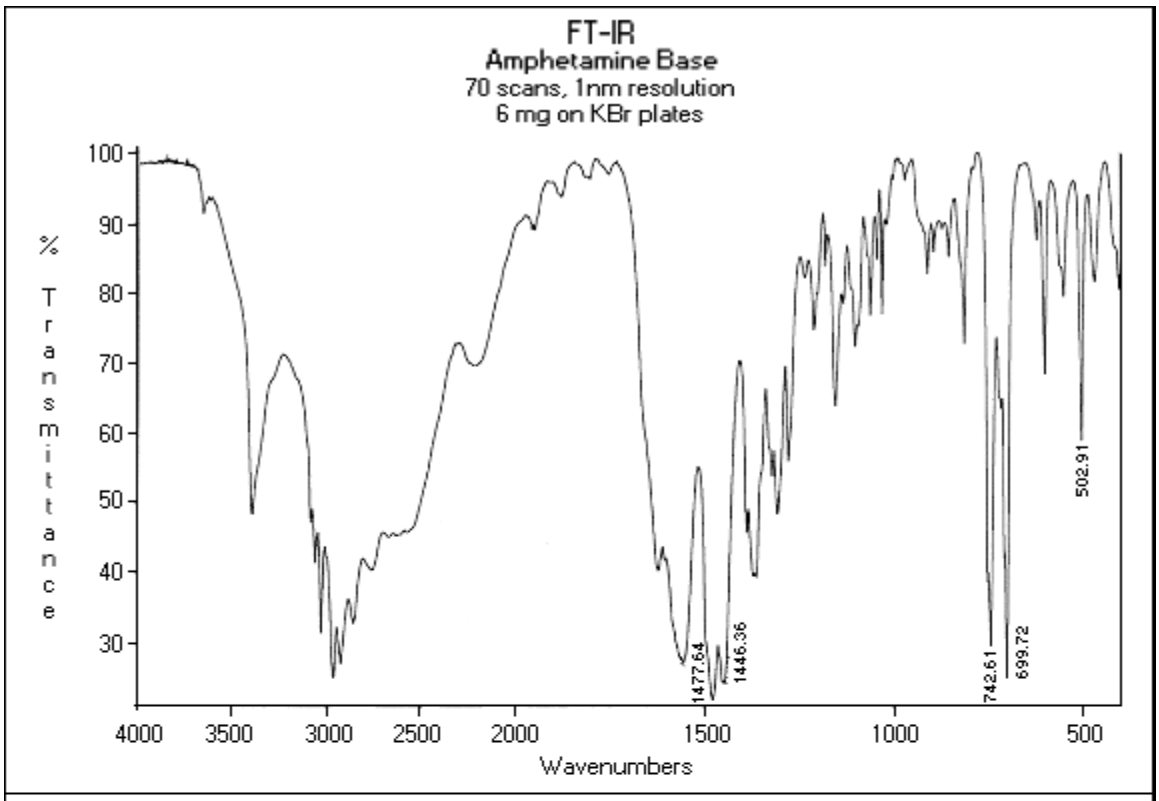
8. ADDITIONAL RESOURCES

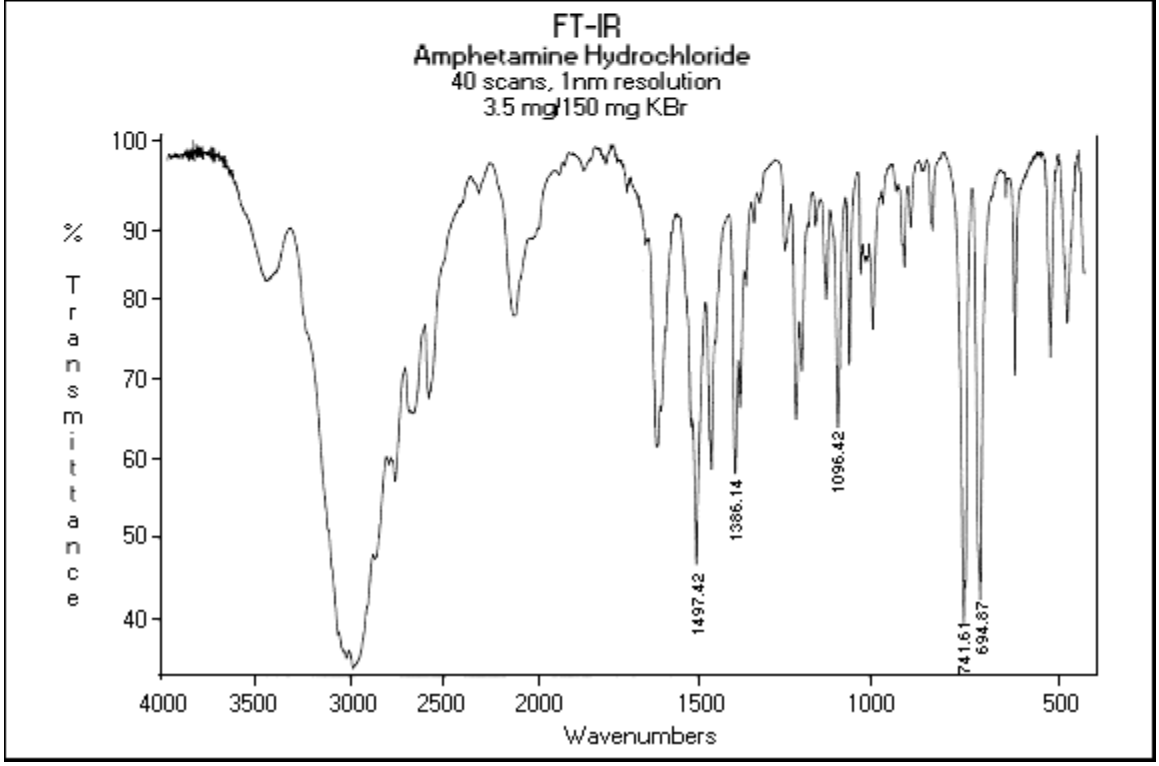
Forendex

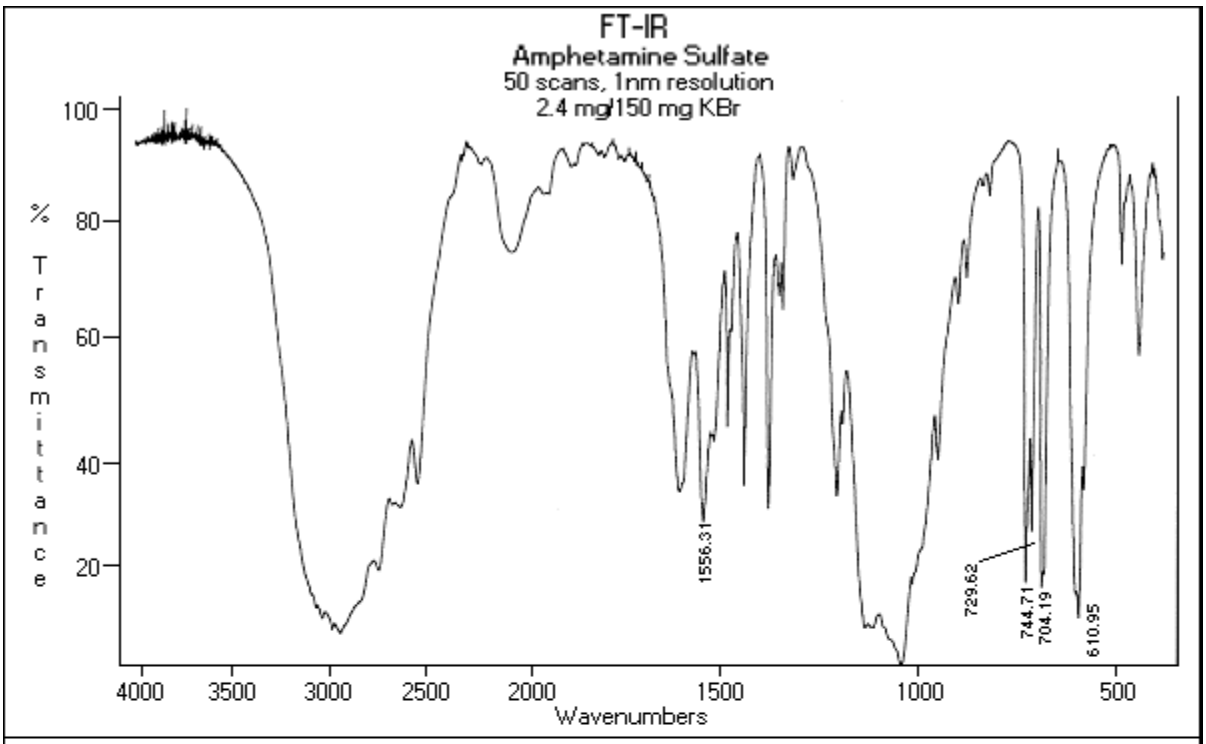
Wikipedia

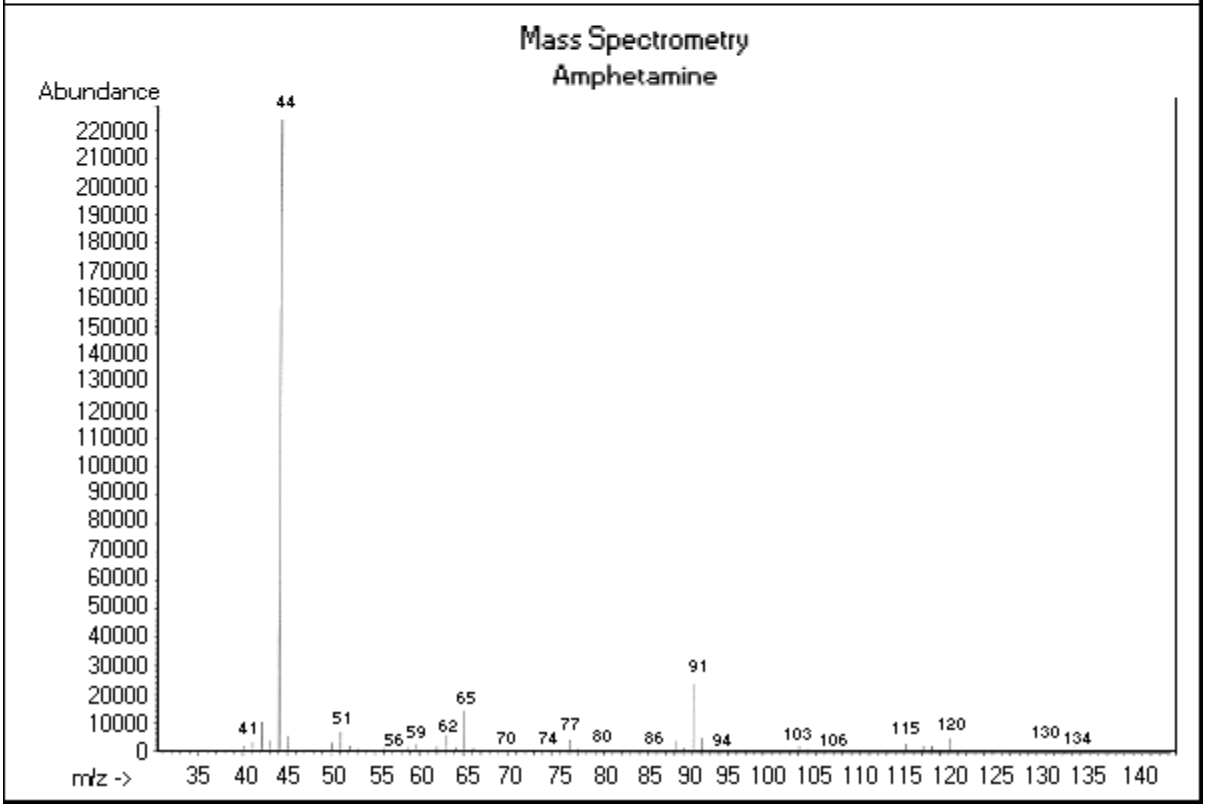

LC-MS (ESI)
Amphetamine
10mM Ammonium Formate
pH 3.7 150V
91.4
92.4
119.4
120.4
136.6
137.5
100
80
60
40
20
0
40
60
80
100
120
140
m/z


100.04
99.5
99.0
98.5
98.0
97.5
97.0
96.5
96.0
95.51
%T
3072
3033
2968
2926
1607
1495
786
Vapor Phase IR
Amphetamine Base
in Chloroform
4000.4
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
748.4
cm-1


FT-IR
Amphetamine Base
70 scans, 1nm resolution
6 mg on KBr plates
% Transmittance
100
90
80
70
60
50
40
30
1477.64
1446.36
742.61
699.72
502.91
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers


FT-IR
Amphetamine Hydrochloride
40 scans, 1nm resolution
3.5 mg/150 mg KBr
% Transmittance
100
90
80
70
60
50
40
1497.42
1386.14
1096.42
741.61
694.87
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers


FT-IR
Amphetamine Sulfate
50 scans, 1nm resolution
2.4 mg/150 mg KBr
% Transmittance
100
80
60
40
20
1556.31
729.62
744.71
704.19
610.95
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers


Mass Spectrometry
Amphetamine
Abundance
m/z ->
44
91
65
51
41
56
59
62
70
74
77
80
86
94
103
106
115
120
130
134

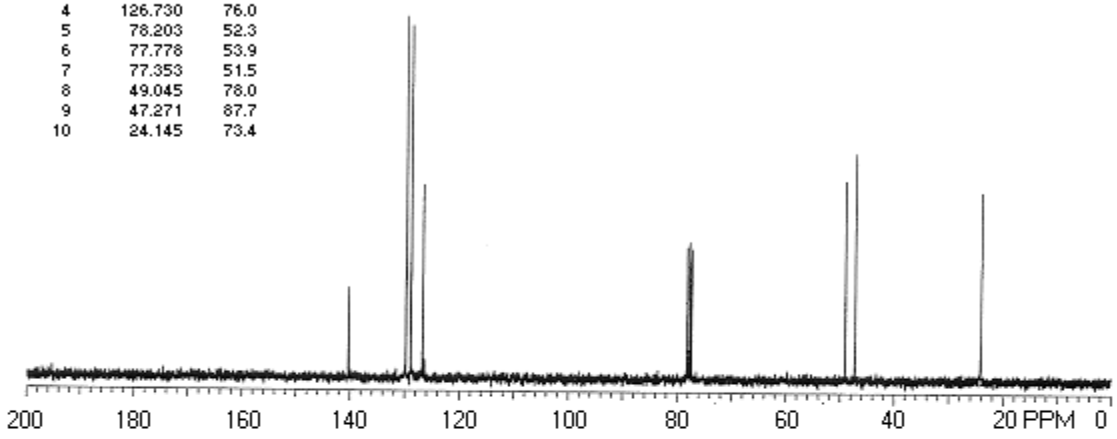
Nuclear Magnetic Resonance (carbon)

Amphetamine Base

50 mg/mL in $CDCL_3$ with TMS

75 MHz

PEAK	PPM	Height
1	140.279	37.2
2	129.811	142.8
3	128.944	143.7
4	126.730	76.0
5	78.203	52.3
6	77.778	53.9
7	77.353	51.5
8	49.045	78.0
9	47.271	87.7
10	24.145	73.4

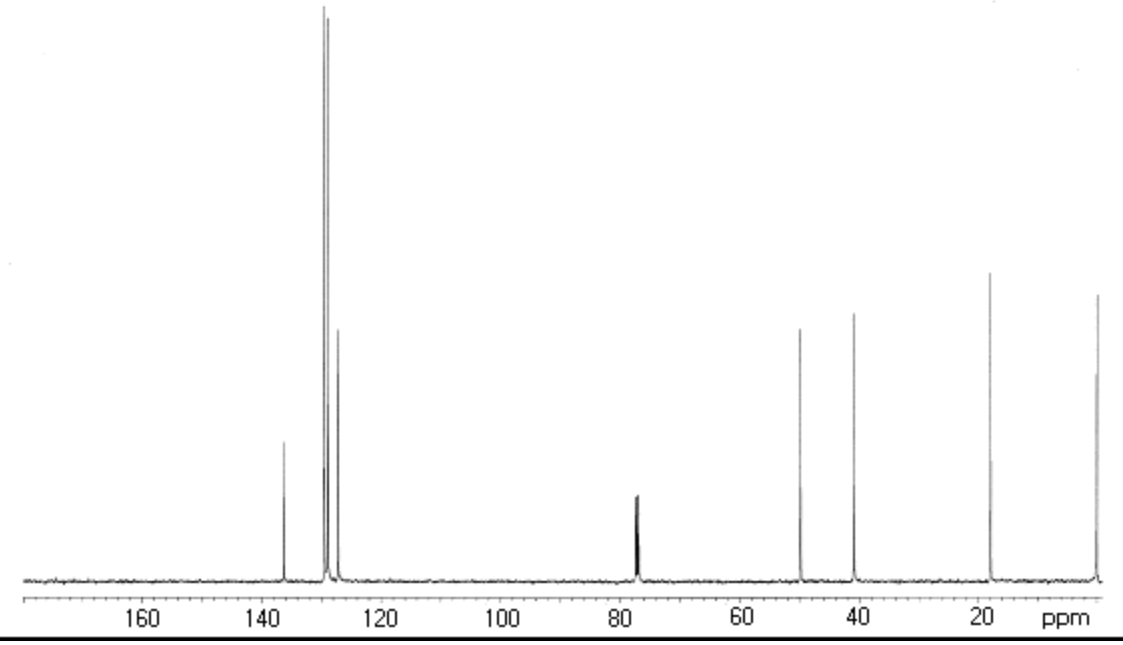


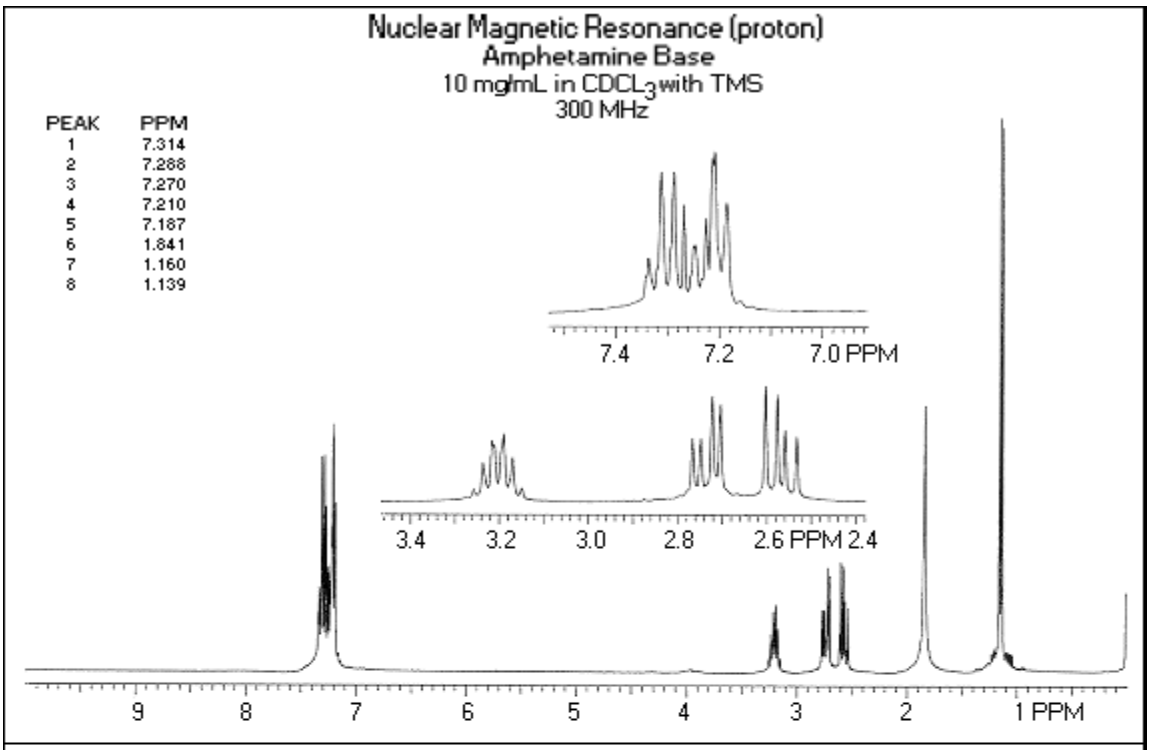
Nuclear Magnetic Resonance (carbon)

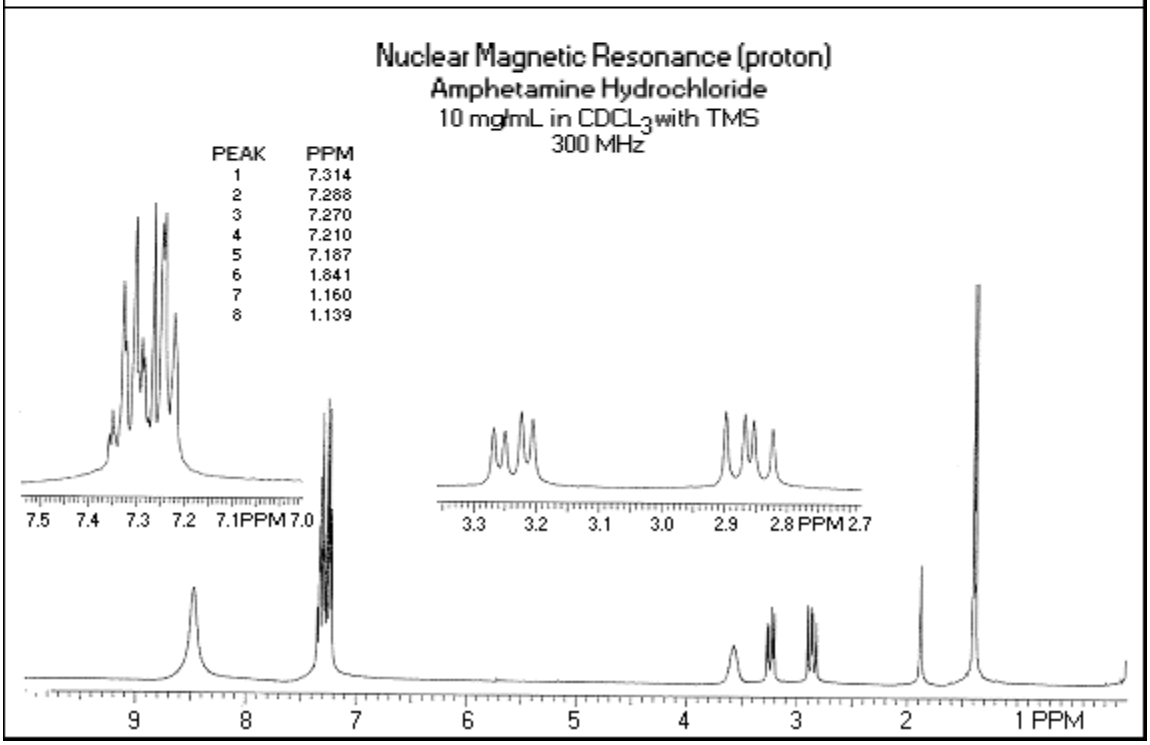
Amphetamine Hydrochloride

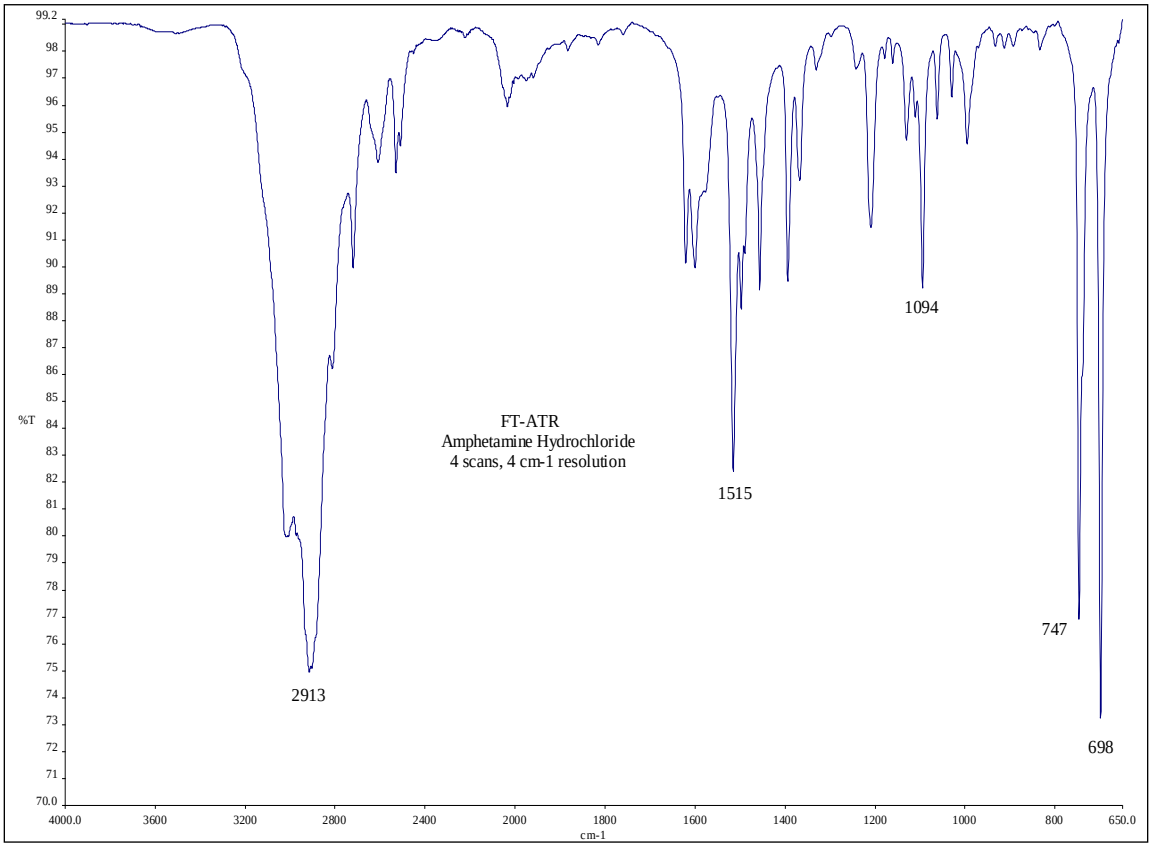
50 mg/mL in $CDCL_3$ with TMS

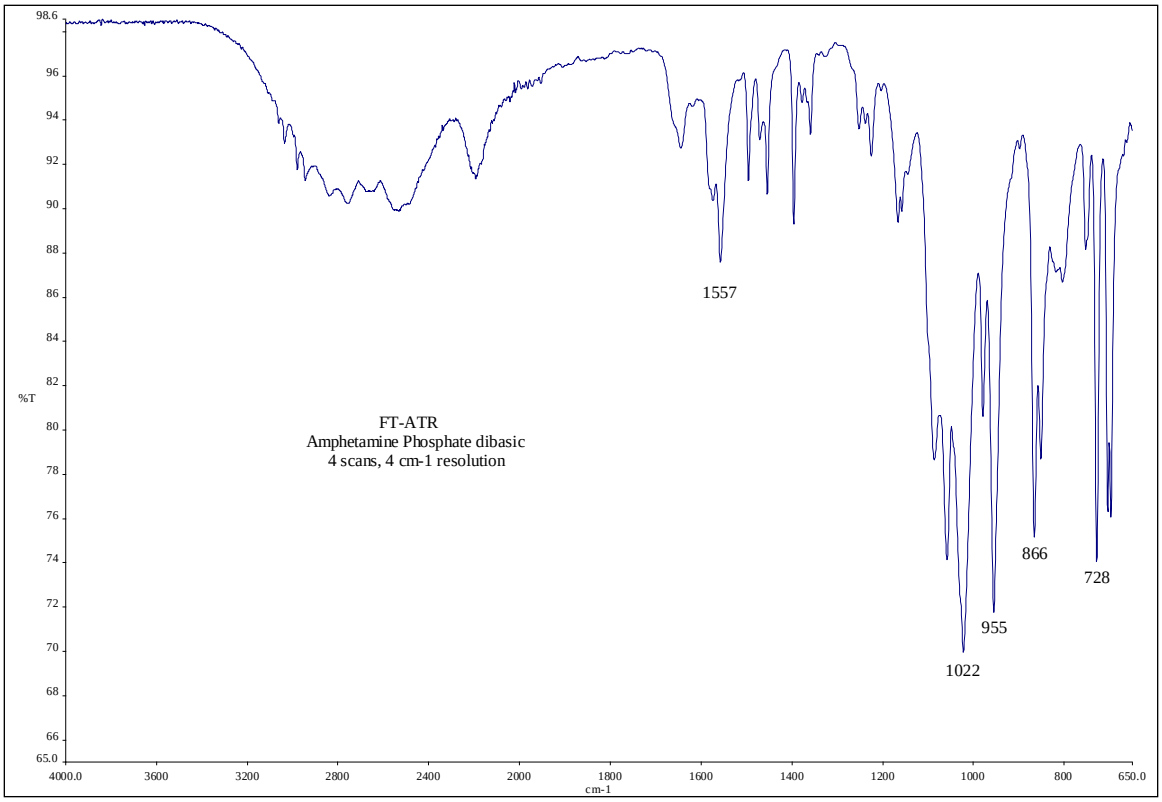
125 MHz

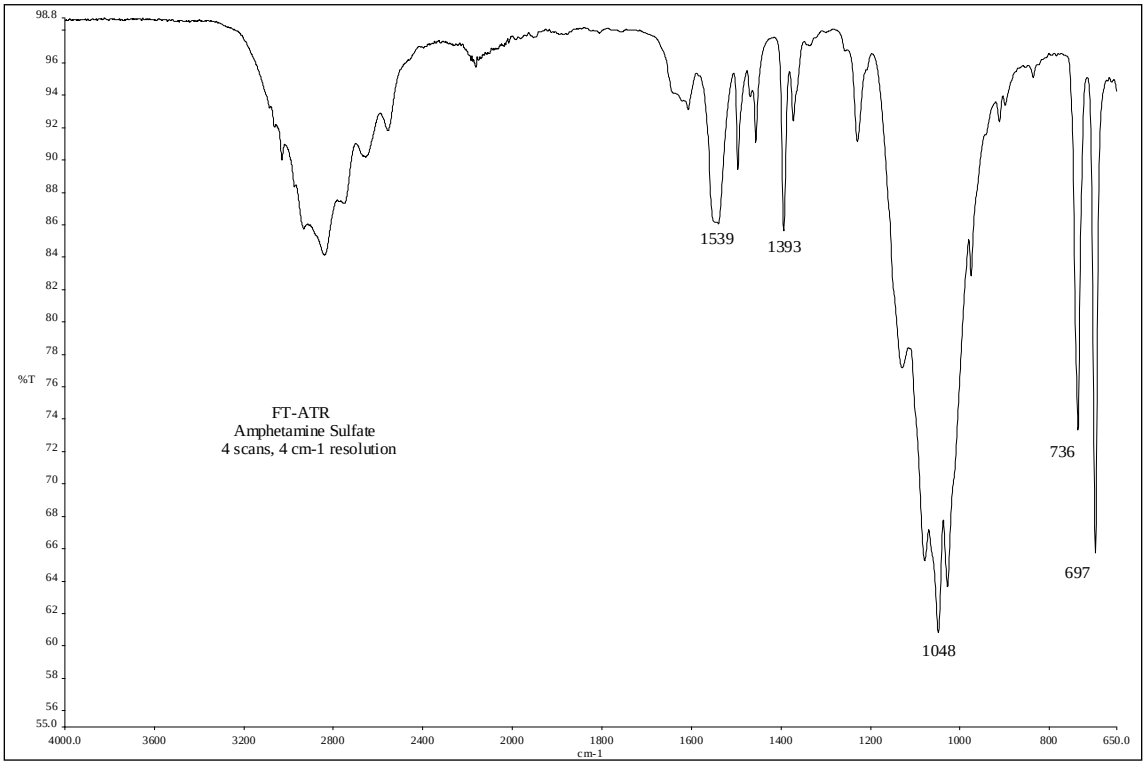


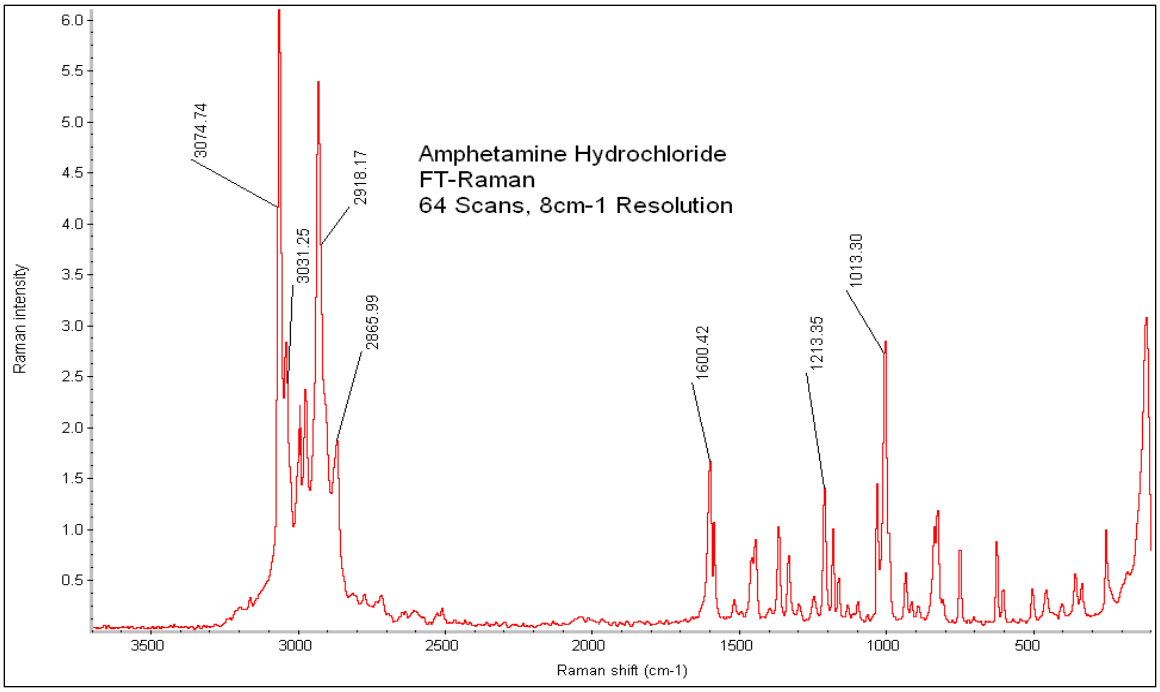

Nuclear Magnetic Resonance (proton)
Amphetamine Base
10 mg/mL in CDCL3 with TMS
300 MHz
PEAK PPM
1 7.314
2 7.288
3 7.270
4 7.210
5 7.187
6 1.841
7 1.160
8 1.139
7.4 7.2 7.0 PPM
3.4 3.2 3.0 2.8 2.6 PPM 2.4
9 8 7 6 5 4 3 2 1 PPM

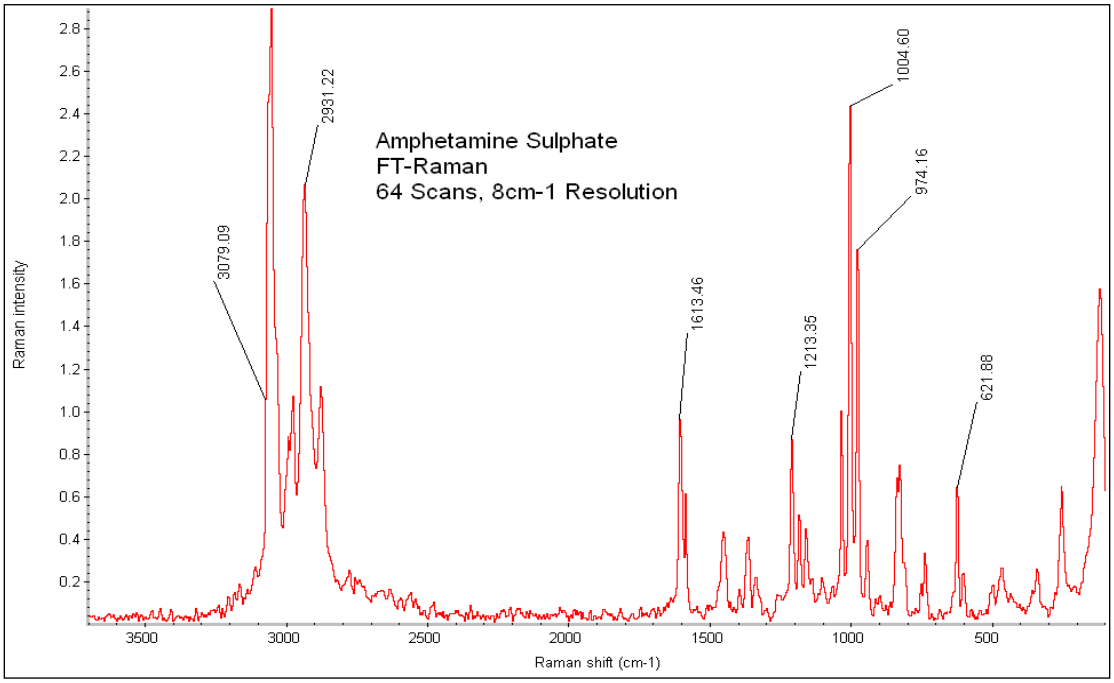

Nuclear Magnetic Resonance (proton)
Amphetamine Hydrochloride
10 mg/mL in CDCL3 with TMS
300 MHz
PEAK PPM
1 7.314
2 7.288
3 7.270
4 7.210
5 7.187
6 1.841
7 1.160
8 1.139
7.5 7.4 7.3 7.2 7.1 PPM 7.0
3.3 3.2 3.1 3.0 2.9 2.8 PPM 2.7
9 8 7 6 5 4 3 2 1 PPM

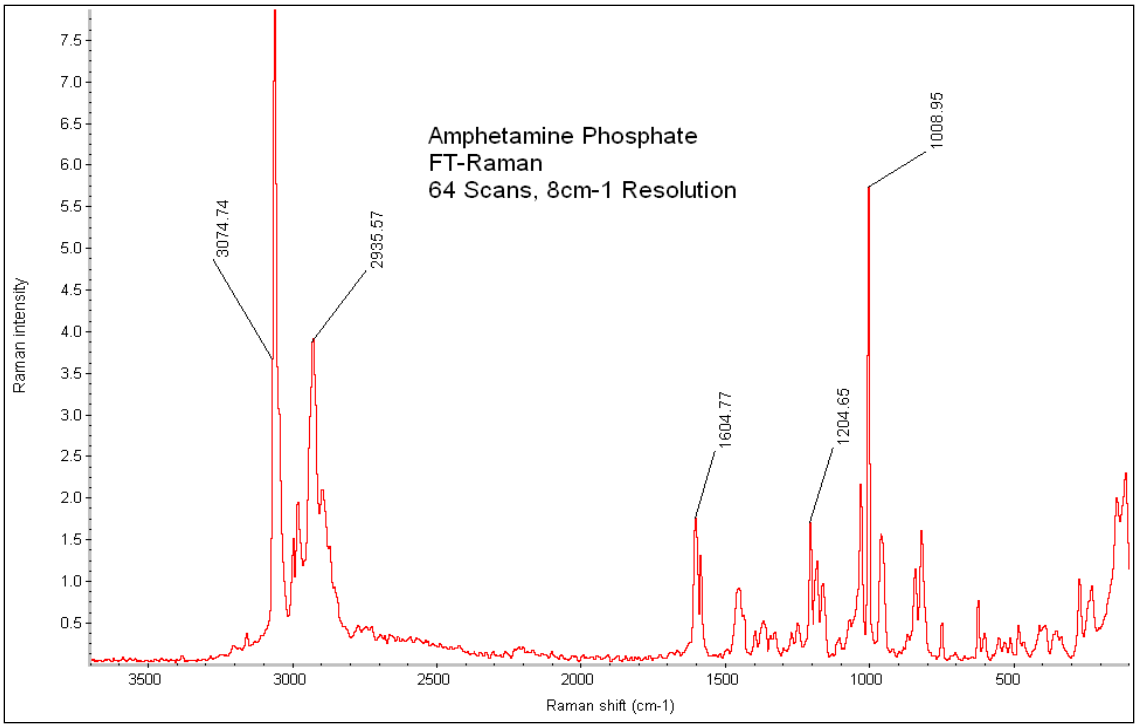

FT-ATR
Amphetamine Hydrochloride
4 scans, 4 cm-1 resolution
%T
cm-1
2913
1515
1094
747
698


FT-ATR
Amphetamine Phosphate dibasic
4 scans, 4 cm-1 resolution
%T
cm-1
1557
1022
955
866
728


%T
cm-1
FT-ATR
Amphetamine Sulfate
4 scans, 4 cm-1 resolution
1539
1393
1048
736
697


Amphetamine Hydrochloride
FT-Raman
64 Scans, 8cm-1 Resolution
Raman intensity
Raman shift (cm-1)
3074.74
2918.17
3031.25
2865.99
1600.42
1213.35
1013.30


Amphetamine Sulphate
FT-Raman
64 Scans, 8cm-1 Resolution
3079.09
2931.22
1613.46
1213.35
1004.60
974.16
621.88
Raman intensity
Raman shift (cm-1)


Amphetamine Phosphate
FT-Raman
64 Scans, 8cm-1 Resolution
3074.74
2935.57
1604.77
1204.65
1008.95
Raman intensity
Raman shift (cm-1)