

TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Forensic Chemistry Standard Operating Procedure Manual

Appendix B: Extraction Guidelines and Procedures

APPENDIX B: EXTRACTION GUIDELINES AND PROCEDURES

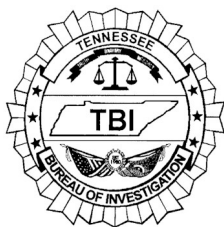
⚠ *All extractions, evaporations, and crystallizations should be performed under a fume hood.*

B.1 General Extraction Guidelines

The TBI FCU uses a wide range of solvents and reagents to extract compounds of interest from various matrices. The appropriate use of the solvents and reagents is extensively covered in the analyst's training program.

Some factors to keep in mind when selecting and using solvents are:

- It is the responsibility of the analyst to understand that controlled substances vary in solubility with regard to different solvents. This property can be used to separate drugs of interest from a sample matrix. References can assist in devising suitable extraction schemes. Using varied, appropriate extraction schemes for unknown substances is an integral part of an analyst's competency and does not require permission for deviation from written procedure.
- Highly polar solutions are destructive to the GC column. Do not run samples that are highly acidic or basic. Special circumstances may dictate that a sample must be run in aqueous solution on a GC column. This practice is highly discouraged, and the analyst should attempt another extraction method if at all possible before injecting an aqueous sample.
- Most controlled substances and pharmaceuticals are compounds that will dissolve in methanol. If the sample is a mixture and co-elution occurs, further extractions may need to be done to isolate the compound of interest if a separation technique is not employed.
- Basic extractions will isolate many controlled substances. The organic layer may then be analyzed on the appropriate instrumentation, used in a presumptive test, or dried down to collect the solid for testing.
- Some controlled substances are insoluble in specific solvents or will not be detected with a particular extraction scheme. If an analyst encounters a complete unknown, multiple solvents or extractions should be performed to ensure false negatives are avoided.
- Compounds of interest can react with certain solvents and reagents. Care must be used by the analyst when choosing extraction schemes.
- Plant or fungal material can be extracted with a one-step soak in methanol, petroleum ether, hexane or other solvent. However, such extractions sometimes contain extraneous compounds that may interfere with analysis. In these cases, a multi-step extraction may be performed.



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B.2 Abbreviations of Extraction Procedures

B → CHCl₃

- make basic and shake into CHCl₃

B → CHCl₃ + HCl

- make basic and shake into CHCl₃, then add a drop of HCl or bubble HCl through CHCl₃

A → CHCl₃

- make acidic and shake into CHCl₃

0.1 N HCl, filter then wash three times with CHCl₃ then B → CHCl₃

- take up in 0.1 N HCl, filter then wash the HCl three times with CHCl₃, discarding the CHCl₃ each time, then make the HCl basic and shake into CHCl₃

CHCl₃ extraction

- shake the sample with CHCl₃ and filter

NH₄OH/CHCl₃ extraction

- shake the sample with ammoniacal CHCl₃ and filter

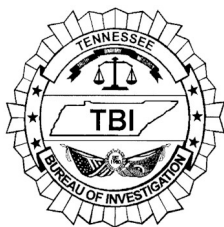
Dilute NaOH, filter, then A → CHCl₃, wash three times with bicarb, then → 0.5 N NaOH then A → CHCl₃

- take up in dilute NaOH, filter then make acidic and extract into CHCl₃, wash the CHCl₃ three times with saturated bicarbonate solution, discarding the bicarbonate, then extract the CHCl₃ with 0.5 N NaOH then make acidic and extract into CHCl₃

B.3 Methods of Extraction for Common Controlled Substances

Alprazolam

- CHCl₃ → filter → wash with H₂O → dry CHCl₃ → wash precipitate with hexane two or three times
- Dichloroethane → filter, evap. → add KBr and wash in filter paper with hexane and run Bicarb → CHCl₃
- B → CHCl₃



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Amphetamine

- B → CHCl₃ + HCl evaporate and crystallize with pet ether
- Biphentamine - take up in 10% HCl, heat 5 min., then extract as above
- Eskatrol - B → CHCl₃ → dil. acetic acid, then extract as above
- Bicarb → CHCl₃
- B → CHCl₃

Anileridine

- B → CHCl₃, run as free base

Barbiturates

- A → CHCl₃
- Carbital - H₂O filter, then A → CHCl₃
- Fiorinal - dil. NaOH, vortex, filter, then A → CHCl₃ wash three times with bicarb. then extract into 0.5N NaOH, then A → CHCl₃
- Tuinal - powder + dil. NaOH + 10 drops saturated KMNO₄, shake well, make acidic with HCl (dropwise) then extract → CHCl₃, this yields amobarbital (if FTIR is not perfect, may repeat above procedure on crystallized material)

Benzphetamine

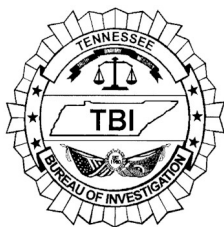
- Didrex - take white center of tab. - CHCl₃ ext. - crystallize with ethyl acetate.
- Didrex - grind up whole tablet, 0.1 N HCl → CHCl₃ (easier to crystallize)
- Bicarb → CHCl₃
- B → CHCl₃

Chlordiazepoxide

- NH₄OH/CHCl₃ ext., crystallize with pet ether, run as free base
- NH₄OH/ CHCl₃ ext., evap. to small volume, add HCl/saturated pet ether, evap. and wash crystals with pet ether, run as HCl
- Limbitrol -
 - H₂O centrifuge, discard H₂O and repeat two times (washing residue with high purity/RO water) then B → CHCl₃
 - 0.1N HCl, filter, wash with CHCl₃ 3x then B → CHCl₃
- Bicarb → CHCl₃
- B → CHCl₃

Clonazepam

- Bicarb → CHCl₃
- B → CHCl₃



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Clorazepate

- 0.5 N H₂SO₄ → CHCl₃ crystallize with pet ether, this gives desmethyldiazepam
- Methanol → filter → evap. (no heat or air) CHCl₃ → filter → evap. (no heat or air), if FTIR is not good, wash with pet ether, grind, rerun (get monopotassium salt)

Cocaine

- NH₄OH/CHCl₃ dry extract, run as free base
- Bicarb → CHCl₃
- B → CHCl₃
- With lidocaine, procaine or caffeine - take up in CHCl₃, filter extract into 0.1 N HCl, take 0.1 N HCl layer, add cobalt thiocyanate + 2-3 drops conc. HCl, shake with CHCl₃, wash CHCl₃ with NH₄OH and discard NH₄OH, then wash CHCl₃ with H₂O to remove NH₄OH, evap. CHCl₃ and run as free base or extract into 0.1 N HCl and repeat above procedure up to two or three more times if necessary
- With lidocaine - wash powder three or four times with dioxane, discard dioxane, take powder 0.1 N HCl, filter then B → CHCl₃ (or take powder after dioxane wash and extract with NH₄OH/CHCl₃)
- With lidocaine or nictotinamide - wash with acetone and filter (cocaine is left in filter paper)
- With nicotinamide - NH₄OH/pet ether, dry extract, run as free base
- With aspirin - H₂O, filter B → CHCl₃ (sodium bicarbonate) wash CHCl₃ twice with saturated sodium bicarbonate solution, evap. CHCl₃
- Cocaine base (with inositol, mannitol, lactose, etc.) - CHCl₃ extraction

Codeine

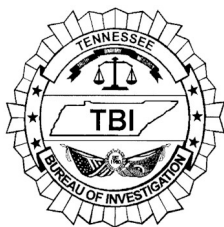
- APC (aspirin, phenacetin, and caffeine) + codeine - 0.1N HCl, filter, wash 3x with CHCl₃ (discard CHCl₃) then B → CHCl₃ or hexane, run as free base, crystallize with pet ether
- Bicarb → CHCl₃
- B → CHCl₃

Diazepam

- CHCl₃ ext., evap., crystallize with pet ether
- Bicarb → CHCl₃
- B → CHCl₃

Diethylpropion

- 0.1 N HCl, filter then B → pet ether, filter, bubble HCl or add a drop of HCl evap.
- B → CHCl₃ + HCl (do not filter, but centrifuge instead)
- B → CHCl₃



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Dihydrocodeine

- Bicarb → CHCl_3
- B → CHCl_3
- Synalgos DC - 0.1 N HCl, filter, wash three times with CHCl_3 (discard CHCl_3), then B → CHCl_3 → dil. acetic acid, make basic → CHCl_3 , evap., crystallize with pet ether

Diphenoxylate

- CHCl_3 ext.
- B → CHCl_3

Ethchlorvynol

- pet ether (wash 4x with H_2O), filter and evap., run on salt plate

Fenfluramine

- CHCl_3 ext.
- Bicarb → CHCl_3
- B → CHCl_3

Flurazepam

- CHCl_3 ext. - crystallize with pet ether
- Bicarb → CHCl_3
- B → CHCl_3

Glutethimide

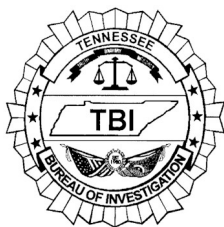
- CHCl_3 ext.

Halazepam

- 0.1N HCl → CHCl_3
- Bicarb → CHCl_3
- B → CHCl_3

Heroin

- CHCl_3 ext.
- 0.1 N HCl → CHCl_3 → 1% tartaric acid (or 1% citric acid) make basic with bicarb. shake → CHCl_3 (may wash with 2.8 N HCl and discard HCl) may be run as base or HCl, crystallize with pet ether
- 0.1 N HCl → CHCl_3
- 2.8 N HCl → CHCl_3 (higher yield than 0.1 N HCl)
- Heroin (with quinine, procaine) - 2.8 N HCl → CHCl_3 → 2% citric acid → add bicarb. → CHCl_3 , run as base



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Hydrocodone

- Hydrocodone with acetaminophen - 0.1 N HCl, wash with CHCl_3 three times make basic $\rightarrow \text{CHCl}_3$
- Bicarb $\rightarrow \text{CHCl}_3$
- B $\rightarrow \text{CHCl}_3$

Hydromorphone

- Dilaudid
 - 0.1 N HCl, filter, basic with bicarb shake into CHCl_3 :Methanol (4:1)
 - $\text{NH}_4\text{OH}/\text{CHCl}_3$, dry extract, crystallize with pet ether
- 0.1 N HCl $\rightarrow$ filter $\rightarrow \text{CHCl}_3$ (discard) $\rightarrow$ basic with bicarb $\rightarrow \text{CHCl}_3$:Methanol (4:1) crystallize with pet ether
- Bicarb $\rightarrow \text{CHCl}_3$
- B $\rightarrow \text{CHCl}_3$

MDA

- CHCl_3 ext.
- B $\rightarrow \text{CHCl}_3$ + HCl evaporate and crystallize with pet ether
- Bicarb $\rightarrow \text{CHCl}_3$
- B $\rightarrow \text{CHCl}_3$

MDMA

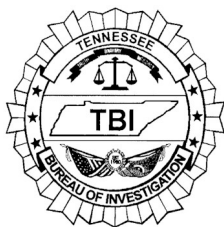
- CHCl_3 ext.
- B $\rightarrow \text{CHCl}_3$ + HCl evaporate and crystallize with pet ether
- Bicarb $\rightarrow \text{CHCl}_3$
- B $\rightarrow \text{CHCl}_3$

Meprobamate

- Scrape some of tablet and run neat on FTIR
- CHCl_3 ext.
- Equagesic - take white layer only- CHCl_3 ext

Mescaline

- B $\rightarrow \text{CHCl}_3$ + HCl
- B $\rightarrow \text{CHCl}_3$



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Methadone

- From liquid - ext. with CHCl_3 then shake CHCl_3 with 0.5 N H_2SO_4 , make basic $\rightarrow$ CHCl_3 bubble HCl evap. and crystallize with ethyl acetate
- B $\rightarrow$ CHCl_3
- Bicarb $\rightarrow$ CHCl_3

Methamphetamine

- CHCl_3 ext.
- B $\rightarrow$ CHCl_3 + HCl
- Bicarb $\rightarrow$ CHCl_3
- B $\rightarrow$ CHCl_3
- Desoxyn - 0.1 N HCl, filter, wash 2x with CHCl_3 (discard CHCl_3) then B $\rightarrow$ pet ether + HCl
- 0.1 N HCl $\rightarrow$ basic with NaOH $\rightarrow$ pet ether + HCl (0.1 N HCl may be washed 3 times with CHCl_3 to eliminate color if needed)
- Cut with nicotinamide -
 - 0.1 N HCl $\rightarrow$ filter $\rightarrow$ wash with CHCl_3 three times (discard CHCl_3) $\rightarrow$ add $\rightarrow$ CHCl_3 $\rightarrow$ dilute acetic acid (2 drops/10 mL), acetic acid + NaOH $\rightarrow$ CHCl_3 + HCl evap.
 - put powder in funnel $\rightarrow$ wash with ether (discard ether) $\rightarrow$ add CHCl_3 $\rightarrow$ evap. CHCl_3 $\rightarrow$ recrystallize with pet ether
 - put powder in funnel $\rightarrow$ wash with acetone (discard acetone), CHCl_3 ext. evap. CHCl_3

Methaqualone

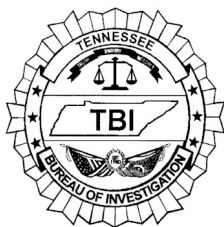
- Quaalude or Sopor - CHCl_3 ext.
- Parest - $\text{NH}_4\text{OH}/\text{CHCl}_3$ ext., run as free base

Methylphenidate

- Ritalin- B $\rightarrow$ CHCl_3 $\rightarrow$ 0.5 N H_2SO_4 make basic $\rightarrow$ CHCl_3 + HCl, evap.
- Bicarb $\rightarrow$ CHCl_3
- B $\rightarrow$ CHCl_3

Methyprylon

- CHCl_3 ext.



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Morphine

- Injectable liquid - 0.5 N H₂SO₄ wash with CHCl₃ (discard CHCl₃) then bicarb → CHCl₃ : Methanol (4:1)
- Morphine sulfate - bicarb → CHCl₃:Methanol (4:1)
- Time release (pf/30) tablet - digest in 0.5 N H₂SO₄ (for 30-45 min), wash two times with CHCl₃, make basic (pH 8.5) 2-3 drops of NH₄OH → CHCl₃:Methanol (4:1)
- Tablets and Time release - high purity/RO water/methanol extraction(equal amounts) water/ethanol extraction(equal amounts) take up in small amount of water

Oxazepam

- NH₄OH/CHCl₃ ext.
- Bicarb → CHCl₃
- B → CHCl₃

Oxycodone

- Percodan - 0.1 N HCl, filter, wash three times with CHCl₃ (discard CHCl₃), then B → CHCl₃
- H₂O → basic with NaOH → CHCl₃ or hexane
- Tylox - NH₄OH/CHCl₃ ext.
- B → CHCl₃
- Bicarb → CHCl₃

Pethidine

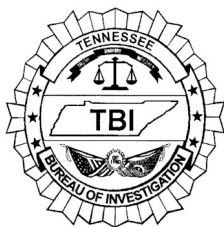
- Demerol - CHCl₃ ext. (heat with dryer to aid crystallization) crystallize with pet ether
- Mepergan Fortis - B → CHCl₃, dil. acetic acid (2 drops in 10 mL), make basic → CHCl₃ + HCl
- Demerol + APC or APAP - 0.5 N H₂SO₄ wash two times with CHCl₃ (discard CHCl₃) then B → CHCl₃ + HCl
- Bicarb → CHCl₃
- B → CHCl₃

PCP

- 0.1 N HCl → CHCl₃
- CHCl₃ ext. (may need to wash with pet ether after adding KBr)
- B → CHCl₃ + HCl
- B → CHCl₃

Pentazocine

- Bicarb → CHCl₃ + HCl
- B → CHCl₃



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Phendimetrazine

- B → CHCl₃ + HCl dry well with dryer before adding pet ether to crystallize
- Plegine - 1% tartaric acid, wash three times with CHCl₃ then B → CHCl₃ + HCl
- Bicarb → CHCl₃
- B → CHCl₃

Phenmetrazine

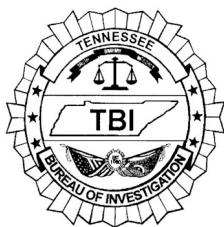
- Preludin tablets - CHCl₃ ext.
- Preludin endurets - 0.1 N HCl filter then B → CHCl₃ + HCl dry well and crystallize with pet ether, may allow to set overnight to crystallize
- 0.1 N HCl, filter, B → CHCl₃ → 0.5 N H₂SO₄, then B → CHCl₃ + HCl, evap.
- Bicarb → CHCl₃
- B → CHCl₃

Phentermine

- B → CHCl₃ + HCl
- CHCl₃ ext.
- Ionamin - 0.1N HCl or 0.5 N H₂SO₄ heat 5 min. Then B → CHCl₃ + HCl
- Bicarb → CHCl₃
- B → CHCl₃

Propoxyphene

- Darvon - NH₄OH/CHCl₃ ext.
- APC -
 - H₂O filter then make basic with bicarb → CHCl₃ → 0.5 N H₂SO₄ wash three times with CHCl₃ (discard CHCl₃) then B → CHCl₃
 - H₂O filter & add approx. 6 drops conc. H₂SO₄ wash 3x with CHCl₃ (discard) then B → CHCl₃
 - 0.5N H₂SO₄ wash three times with CHCl₃ (discard CHCl₃) filter then bicarb → hexane
- Napsylate + acetaminophen -
 - CHCl₃, centrifuge, remove liquid (discard residue) and wash two times with dil. NaOH (centrifuge each time and discard NaOH) then extract into 0.5 N H₂SO₄ then B → CHCl₃
 - dissolve tablet in H₂O, filter, make basic with bicarb. and shake into hexane (or pet ether), run as free base
 - pet ether wash with H₂O + 2 drops NH₄OH, discard H₂O, evaporate, crystallize with pet ether
- With caffeine- NH₄OH + hexane (filter) extract into 0.5N H₂SO₄, wash three times with CHCl₃, make basic → CHCl₃



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- Bicarb → CHCl_3
- B → CHCl_3

Secobarbital

- A → CHCl_3 , evaporate with cool air stream, avoid heat

B.4 Alternate Non-Aqueous Organic Ratio (ANOR) Extraction Procedure

B.4.1 Application

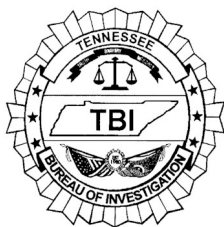
The ANOR extraction procedure utilizes an organic solvent saturated with ammonium hydroxide or hydrochloric acid. The NH_4OH or HCl is mixed with the desired solvent at a ratio of approximately one part acid or base to ten parts solvent. If the NH_4OH or HCl is lighter than the solvent, such as the case with chloroform, a separatory funnel is useful for storing the solvent until ready for use. If the NH_4OH or HCl is heavier than the desired solvent, such as the case with petroleum ether and hexane, a reagent bottle can be utilized for storing the solvent, and the upper layer is removed with a disposable pipette.

B.4.2 Procedure

- Determine proper solvent system.
- Extract the powder in one of two ways:
 - Place drug sample as a powder in filter paper - elute with proper solvent system and catch the eluent.
 - Place the powder and solvent system in a test tube – vortex and filter.
- Evaporate the solvent to obtain the free acid, base, ion pair, or salt (if HCl is added to elute).

B.4.3 Drugs Extracted Using NH_4OH -Saturated CHCl_3

- Benzodiazepines
- Benzphetamine
- Chlordiazepoxide
- Cocaine
- Diazepam
- Flurazepam
- Hydromorphone
- Lorazepam
- Methaqualone
- Morphine
- Phentermine



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B.4.4 Drugs Extracted Using NH_4OH -Saturated Hexanes

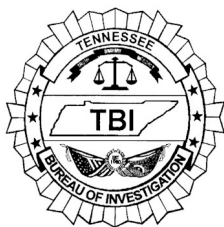
- 3,4-Methylenedioxyamphetamine
- Amphetamine
- Chlorphentermine
- Clortermine
- Codeine w/ Acetaminophen
- Diazepam
- Diethylpropion
- Ephedrine
- Hydrocodone
- Meperidine
- Methamphetamine
- Oxycodone w/ Acetaminophen
- Phentermine
- Phenylpropanolamine
- Propoxyphene

B.4.5 Drugs Extracted Using HCl -Saturated CHCl_3

- Barbiturates (Most)
- Cocaine
- Diazepam
- Diphenylhydantoin
- Heroin
- Meperidine
- Pentazocine
- Pentobarbital
- Phencyclidine
- Phenmetrazine
- Phenobarbital
- Secobarbital

B.4.6 Drugs Extracted Using NH_4OH -Saturated Petroleum Ether

- Cocaine – w/ Nicotinamide
- Cocaine – Lidocaine mixtures may be extracted by first washing with 1,4-dioxane to obtain lidocaine, then with NH_4OH saturated petroleum ether to obtain cocaine
- Diazepam
- Methamphetamine – Add HCl to form the hydrochloride salt before extraction



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B.4.7 *Drugs Extracted Using HCl-Saturated Diethyl Ether/Hexane (4:1)*

- Butalbital – from a mixture of caffeine, acetaminophen and butalbital

B.4.8 *Drugs Extracted Using NH₄OH-Saturated CHCl₃/Hexane (4:1)*

- Chlordiazepoxide – from a mixture of chlordiazepoxide and amitriptyline

B.5 *References*

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Moffat, A.C., *Clarke's Isolation and Identification of Drugs*, 2nd Ed., The Pharmaceutical Press, London, 1986.

Alternate Non-Aqueous Organic Ratio (ANOR) Extraction Procedure, *Microgram* Vol. XVI No.1 January 1983