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.

B.2 Abbreviations of Extraction Procedures

B → $CHCl_3$

- make basic and shake into $CHCl_3$

B → $CHCl_3$+ HCl

- make basic and shake into $CHCl_3$, then add a drop of HCl or bubble HCl through $CHCl_3$

A → $CHCl_3$

- make acidic and shake into $CHCl_3$

0.1 N HCl, filter then wash three times with $CHCl_3$ then B →$CHCl_3$

- take up in 0.1 N HCl, filter then wash the HCl three times with $CHCl_3$, discarding the $CHCl_3$ each time, then make the HCl basic and shake into $CHCl_3$

$CHCl_3$ extraction

- shake the sample with $CHCl_3$ and filter

$NH_4OH/CHCl_3$ extraction

- shake the sample with ammoniacal $CHCl_3$ and filter

Dilute NaOH, filter, then A → CHCl3, wash three times with bicarb, then → 0.5 N NaOH then A →$CHCl_3$

- take up in dilute NaOH, filter then make acidic and extract into $CHCl_3$, wash the $CHCl_3$ three times with saturated bicarbonate solution, discarding the bicarbonate, then extract the $CHCl_3$ with 0.5 N NaOH then make acidic and extract into $CHCl_3$

B.3 Methods of Extraction for Common Controlled Substances

Alprazolam

- $CHCl_3$→filter→wash with H_2O→dry $CHCl_3$→wash precipitate with hexane two or three times
- Dichloroethane→filter, evap. →add KBr and wash in filter paper with hexane and run Bicarb→$CHCl_3$
- B→ $CHCl_3$

Amphetamine

- B→ $CHCl_3$ + HCl evaporate and crystallize with pet ether
- Biphetamine - take up in 10% HCl, heat 5 min., then extract as above
- Eskatrol - B→ $CHCl_3$→dil. acetic acid, then extract as above
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Anileridine

- B→ $CHCl_3$, run as free base

Barbiturates

- A→ $CHCl_3$
- Carbital - H_2O filter, then A→ $CHCl_3$
- Fiorinal - dil. NaOH, vortex, filter, then A→ $CHCl_3$ wash three times with bicarb. then extract into 0.5N NaOH, then A→ $CHCl_3$
- Tuinal - powder + dil. NaOH + 10 drops saturated $KMNO_4$, shake well, make acidic with HCl (dropwise) then extract→ $CHCl_3$, this yields amobarbital (if FTIR is not perfect, may repeat above procedure on crystallized material)

Benzphetamine

- Didrex - take white center of tab. - $CHCl_3$ ext. - crystallize with ethyl acetate.
- Didrex - grind up whole tablet, 0.1 N HCl→ $CHCl_3$ (easier to crystallize)
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Chlordiazepoxide

- $NH_4OH/CHCl_3$ ext., crystallize with pet ether, run as free base
- NH_4OH/ $CHCl_3$ ext., evap. to small volume, add HCl/saturated pet ether, evap. and wash crystals with pet ether, run as HCl
- Limbitrol -
    - H_2O centrifuge, discard H_2O and repeat two times (washing residue with high purity/RO water) then B→ $CHCl_3$
    - 0.1N HCl, filter, wash with $CHCl_3$ 3x then B→ $CHCl_3$
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Clonazepam

- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Clorazepate

- 0.5 N H_2SO_4→ $CHCl_3$ crystallize with pet ether, this gives desmethyldiazepam
- Methanol→filter→ evap. (no heat or air) $CHCl_3$→ filter→evap. (no heat or air), if FTIR is not good, wash with pet ether, grind, rerun (get monopotassium salt)

Cocaine

- $NH_4OH/CHCl_3$ dry extract, run as free base
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$
- With lidocaine, procaine or caffeine - take up in $CHCl_3$, filter extract into 0.1 N HCl, take 0.1 N HCl layer, add cobalt thiocyanate + 2-3 drops conc. HCl, shake with $CHCl_3$, wash $CHCl_3$ with NH_4OH and discard NH_4OH, then wash $CHCl_3$ with H_2O to remove NH_4OH, evap. $CHCl_3$ 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→ $CHCl_3$ (or take powder after dioxane wash and extract with $NH_4OH/CHCl_3$)
- With lidocaine or nictotinamide - wash with acetone and filter (cocaine is left in filter paper)
- With nicotinamide - NH_4OH/pet ether, dry extract, run as free base
- With aspirin - H_2O, filter B→ $CHCl_3$ (sodium bicarbonate) wash $CHCl_3$ twice with saturated sodium bicarbonate solution, evap. $CHCl_3$
- Cocaine base (with inositol, mannitol, lactose, etc.)- $CHCl_3$ extraction

Codeine

- APC (aspirin, phenacetin, and caffeine) + codeine - 0.1N HCl, filter, wash 3x with $CHCl_3$ (discard $CHCl_3$) then B→ $CHCl_3$ or hexane, run as free base, crystallize with pet ether
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Diazepam

- $CHCl_3$ ext., evap., crystallize with pet ether
- Bicarb → $CHCl_3$
- B→ $CHCl_3$

Diethylpropion

- 0.1 N HCl, filter then B→pet ether, filter, bubble HCl or add a drop of HCl evap.
- B→ $CHCl_3$ + HCl (do not filter, but centrifuge instead)
- B→ $CHCl_3$

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

Hydrocodone

- Hydrocodone with acetaminophen - 0.1 N HCl, wash with $CHCl_3$ three times make basic→ $CHCl_3$
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Hydromorphone

- Dilaudid
    - 0.1 N HCl, filter, basic with bicarb shake into $CHCl_3$:Methanol (4:1)
    - $NH_4OH/CHCl_3$, dry extract, crystallize with pet ether
- 0.1 N HCl→filter→ $CHCl_3$ (discard)→basic with bicarb→ $CHCl_3$:Methanol (4:1) crystallize with pet ether
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

MDA

- $CHCl_3$ ext.
- B→ $CHCl_3$ + HCl evaporate and crystallize with pet ether
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

MDMA

- $CHCl_3$ ext.
- B→ $CHCl_3$ + HCl evaporate and crystallize with pet ether
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Meprobamate

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

Mescaline

- B→ $CHCl_3$ + HCl
- B→ $CHCl_3$

Methadone

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

Methamphetamine

- $CHCl_3$ ext.
- B→ $CHCl_3$ + HCl
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$
- Desoxyn - 0.1 N HCl, filter, wash 2x with $CHCl_3$ (discard $CHCl_3$) then B→pet ether + HCl
- 0.1 N HCl→basic with NaOH→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→filter→wash with $CHCl_3$ three times (discard $CHCl_3$)→add → $CHCl_3$→dilute acetic acid(2 drops/10 mL), acetic acid + NaOH→ $CHCl_3$ + HCl evap.
    - put powder in funnel→wash with ether (discard ether)→add $CHCl_3$→evap. $CHCl_3$→recrystallize with pet ether
    - put powder in funnel→wash with acetone (discard acetone), $CHCl_3$ ext. evap. $CHCl_3$

Methaqualone

- Quaalude or Sopor - $CHCl_3$ ext.
- Parest - $NH_4OH/CHCl_3$ ext., run as free base

Methylphenidate

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

Methyprylon

- $CHCl_3$ ext.

Morphine

- Injectible liquid - 0.5 N H_2SO_4 wash with $CHCl_3$ (discard $CHCl_3$) then bicarb → $CHCl_3$: Methanol (4:1)
- Morphine sulfate - bicarb→ $CHCl_3$:Methanol (4:1)
- Time release (pf/30) tablet - digest in 0.5 N H_2SO_4 (for 30-45 min), wash two times with $CHCl_3$, make basic (pH 8.5) 2-3 drops of NH_4OH→ $CHCl_3$: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

- $NH_4OH/CHCl_3$ ext.
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Oxycodone

- Percodan - 0.1 N HCl, filter, wash three times with $CHCl_3$ (discard $CHCl_3$), then B→ $CHCl_3$
- H_2O→basic with NaOH→ $CHCl_3$ or hexane
- Tylox - $NH_4OH/CHCl_3$ ext.
- B→ $CHCl_3$
- Bicarb→ $CHCl_3$

Pethidine

- Demerol - $CHCl_3$ ext. (heat with dryer to aid crystallization) crystallize with pet ether
- Mepergan Fortis - B→$CHCl_3$, dil. acetic acid (2 drops in 10 mL), make basic→CHCl3 + HCl
- Demerol + APC or APAP - 0.5 N H_2SO_4 wash two times with $CHCl_3$ (discard $CHCl_3$) then B→ $CHCl_3$ + HCl
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

PCP

- 0.1 N HCl→ $CHCl_3$
- $CHCl_3$ ext. (may need to wash with pet ether after adding KBr)
- B→ $CHCl_3$ + HCl
- B→ $CHCl_3$

Pentazocine

- Bicarb → $CHCl_3$ + HCl
- B→ $CHCl_3$

Phendimetrazine

- B→ $CHCl_3$ + HCl dry well with dryer before adding pet ether to crystallize
- Plegine - 1% tartaric acid, wash three times with $CHCl_3$ then B→ $CHCl_3$ + HCl
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Phenmetrazine

- Preludin tablets - $CHCl_3$ ext.
- Preludin endurets - 0.1 N HCl filter then B→ $CHCl_3$ + HCl dry well and crystallize with pet ether, may allow to set overnight to crystallize
- 0.1 N HCl, filter, B→ $CHCl_3$→0.5 N H_2SO_4, then B→ $CHCl_3$ + HCl, evap.
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Phentermine

- B→ $CHCl_3$ + HCl
- $CHCl_3$ ext.
- Ionamin - 0.1N HCl or 0.5 N H_2SO_4 heat 5 min. Then B→ $CHCl_3$ + HCl
- Bicarb→ $CHCl_3$
- B→ $CHCl_3$

Propoxyphene

- Darvon - $NH_4OH/CHCl_3$ ext.
- APC -
    - H_2O filter then make basic with bicarb→ $CHCl_3$→0.5 N H_2SO_4 wash three times with $CHCl_3$ (discard $CHCl_3$) then B→ $CHCl_3$
    - H_2O filter & add approx. 6 drops conc. H_2SO_4 wash 3x with $CHCl_3$ (discard) then B→ $CHCl_3$
    - 0.5N H_2SO_4 wash three times with $CHCl_3$ (discard $CHCl_3$) filter then bicarb → hexane
- Napsylate + acetaminophen -
    - $CHCl_3$, centrifuge, remove liquid (discard residue) and wash two times with dil. NaOH (centrifuge each time and discard NaOH) then extract into 0.5 N H_2SO_4 then B→ $CHCl_3$
    - dissolve tablet in H_2O, filter, make basic with bicarb. and shake into hexane (or pet ether), run as free base
    - pet ether wash with H_2O + 2 drops NH_4OH, discard H_2O, evaporate, crystallize with pet ether
- With caffeine- NH_4OH + hexane (filter) extract into 0.5N H_2SO_4, wash three times with $CHCl_3$, make basic→ $CHCl_3$

- 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

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

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 NH_4OH-Saturated $CHCl_3$/Hexane (4:1)

- Chlordiazepoxide – from a mixture of chlordiazepoxide and amitriptyline

B.5 References

Clarke, E.G.C., Ed*., Isolation and Identification of Drugs*, Vol. 1, The Pharmaceutical Press, London, 1971.

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