Western Regional Laboratory
Drug Chemistry Procedure Manual
Effective Date: October 21, 1999

Name of Procedure:

Ultraviolet Spectroscopy
Cecil Ultraviolet Spectrophotometer CE 3021

Suggested Uses:

1. Preliminary qualitative analysis of unknown substances (liquid or solid).

2. Quantitative analysis of known compounds.

Apparatus Needed to Perform Procedure Including Preparation of Reagent:

Cecil Ultraviolet Spectrophotometer CE 3021
Fume hood
Gloves
Eye protection
Laboratory coat
Graduated cylinder
Storage container
Dispensing bottle
Hydrochloric acid (concentrated)
Distilled - deionized water
Funnel
Quartz UV cuvette
Volumetric flasks
Pipettes
Spatula

Formula for Preparing Reagent:

1. Concentrated hydrochloric acid is 12 N. Use a ratio of 1 mL HCl_{conc} to 250 mL distilled water to obtain a 0.05 N HCl solution.

2. Nearly fill the storage container with water and add the appropriate amount of HCl.

3. Mix well, then fill a squeeze bottle with the solution.

4. Label both containers with “0.05 N HCl”, the date, and preparer’s initials.

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Expiration Date of Chemical Reagent:

The 0.05 N HCl solution has an indefinite shelf life if properly stored in an airtight reagent bottle.

Operational Procedures for Using the UV/Vis Spectrophotometer:

1. General Start-up of Instrument:

   a. With compartment door closed, switch UV instrument and printer on.
   b. When instrument initialization is completed (wait 15 minutes).
   c. Press **SCAN** for parameter settings. Select **Store Baseline** and press ENTER. Instrument will load default file. If necessary, change settings using the instruction on screen use (UP-DOWN keys).
   d. Check that exterior of cuvette is clean. Use methanol on Kimwipe® to remove water and fingerprints from clear sides of cuvette. Handle only frosted sides.
   e. Fill a clean cuvette within ¼ inch of top with the HCl solution.
   f. Place cuvette in cell holder nearest the operator, with cuvette letters facing left (toward detector).
   g. Close door completely and press **RUN** to run background.
   h. A clean cuvette is now ready for qualitative or quantitative analysis.

2. **Calibration Check of Cecil CE 3021:**

   a. Load the following parameters (may store as a file):
      1. Scan range: 700 - 210
      2. Slit width: 1.0nm
      3. Absorbance range: 0 - 1.0A
      4. Scan speed: medium or slow
      5. Wavelength scale: 20nm/cm
   b. While cell is empty, run **BASELINE**.
   c. Place standard (Holmium Oxide Solution) into cell holder.
   d. Press **RUN/PRINT** to print scan.
   f. Press **RUN** to print scan of standard.

Calibration Check of Cecil CE 3021 (continued):

NOTE: The monthly calibration using the Holmium oxide standard will be filed and maintained by the UV Coodinator as set forth in the Drug Chemistry Policy and Procedure Manual.

3. Qualitative Analysis:

a. Place 1 mg (tip of spatula) of solid sample, or a small drop of liquid, into cuvette of solution (from above background run).
b. Cover cuvette and invert several times to mix.
c. Place cuvette back into cell holder as above, and close door.
d. Set “Abs.0" to reset absorbance to zero, if desired.
e. Press “RUN” and observe sample absorption.
f. Readjust parameters (absorbance range) or sample concentration as necessary to obtain a scan with peak maxima close to 1 A (desired range is 0.5 A - 1.0 A).
g. Press “RUN/Print, to obtain a hardcopy of sample absorbance.
h. Remove cuvette and rinse well with tap water for the next user.

4. General Quantitation of Known Single Drug (No Interfering Analytes):

a. Prepare a calibration curve of the standard to obtain the absorptivity (a or ε). Make four or five solutions of standard at concentrations 0.1 - 1.0 mg/mL.

Option 1

1. For each solution: accurately measure a known amount of standard (10 - 100 mg) into a 100-mL volumetric flask (or equivalent ratio).

2. Carefully fill to the mark with 0.05 N HCl and mix well.

4. General Quantitation of Known Single Drug (No Interfering Analytes) (cont.):

Option 2 (Serial dilutions)

1. Make the most concentrated standard solution as instructed in "option 1", then take a known aliquot (10 - 80 mL) and dilute with 0.05 N HCl in a 100-mL volumetric flask (or equivalent ratio).

2. Repeat diluting different aliquot amounts in separate flasks so that a range of standard concentrations is established.

3. Run each standard dilution separately, thoroughly rinsing the cuvette between each solution, and record the absorbance for each at the same peak wavelength.

4. Plot absorbance (y) as a function of concentration (x).

5. Obtain the slope of the linear portion of the curve ($A/c = \varepsilon$ when $b = 1$ cm, c = moles/L) or ($A/c = a$ when $b = 1$ cm, c = g/L).

b. Prepare a solution of the sample and quantitate.

1. Accurately measure a known amount of sample into a volumetric flask (approximately 50 mg [or other appropriate amount] into a 100-mL flask or equivalent ratio).

2. Dissolve to the mark with 0.05 N HCl and mix well.

3. Scan sample and record absorbance at the same peak wavelength as the standards.

4. Rinse the cuvette with several aliquots of the sample solution.
   a. Fill the cuvette with the same solution.
   b. Prepare cuvette and scan sample as before.

5. Use $A/\varepsilon = c$ and $c_{drug}/c_{sample} \times 100$ = %drug in sample, to calculate concentration of the analyte.

5. General Quantitation of Known Drug Mixtures:

a. You must know all the absorbing species in the sample.
   $A_T = A_1 + A_2 + ... + A_N = a_1bc_1 + a_2bc_2 + ... + a_Nbc_N = \varepsilon_1c_1 + \varepsilon_2c_2 + ... + \varepsilon_Nc_N$
b. Prepare a calibration curve of each individual standard to obtain the absorptivities at each wavelength (ε_{1-N} at N wavelengths for N analytes).
c. Make four or five solutions of each standard at concentrations 0.1 - 1.0 mg/mL.

Option 1

1. For each solution: accurately measure a known amount of standard (10 - 100 mg) into a 100-mL volumetric flask (or equivalent ratio).

2. Dilute to the mark with 0.05 N HCl and mix well.

Option 2 (Serial dilutions)

1. Make the most concentrated standard solution as instructed in “option 1", then take a known aliquot (10 - 80 mL) and dilute with 0.05 N HCl in a 100-mL volumetric flask (or equivalent ratio).

2. Repeat, diluting successive aliquots in separate flasks so that a range of standard concentrations is established.

3. Run each standard dilution separately, thoroughly rinsing the cuvette between each solution, and record the absorbance for each standard at the same peak wavelengths. The most accurate data is at wavelengths where the molar absorptivity differences are greatest (e.g.: $\lambda'_{max} = \lambda''_{min}$).

4. Plot absorbance (y) as a function of concentration (x) for each standard.

5. Obtain the slope of the linear portion of the curve ($A/c = \varepsilon$ when $b = 1$ cm) for each standard.

d. Prepare a solution of the sample to be quantitated.

1. Measure a known amount of sample into a volumetric flask (approximately 50 mg (or other appropriate amount) into a 100-mL flask or equivalent ratio).

2. Dissolve to the mark with 0.05 N HCl and mix well.

Option 2 (Serial dilutions)(continued)

e. Scan the sample and record the total absorbance at the same peak wavelengths as the standards.
f. Use $A' = \varepsilon'_1 c_1 + \varepsilon'_2 c_2 + ... + \varepsilon'_N c_N$ at λ', $A'' = \varepsilon''_1 c_1 + \varepsilon''_2 c_2 + ... + \varepsilon''_N c_N$ at λ'', to $A^N = \varepsilon^N_1 c_1 + \varepsilon^N_2 c_2 + ... + \varepsilon^N_N c_N$ at λ^N, to calculate concentrations of the analytes.

Quantitation Calculation Examples Using Specific Drugs:

4a. **Single-drug quantitation** using an empirically-derived molar absorptivity for cocaine:

Using **option 1** to make standard solutions:

cocaine base (g) diluted to 100 mL soln	cocaine HCl soln (mg/mL)	A at 275.4 nm
0.0000	0.000	0.0000
0.0112	0.100	0.3757
0.0448	0.400	1.4854
0.0672	0.600	2.1937
0.0896	0.800	2.8723

slope = A/c = a. At 0.900 A, c = 0.246 mg/mL; therefore, (0.900 A)/(0.246 mg/mL) = 3.658 A·mL/mg.
For cocaine base with a molecular weight of 303.3 g/mol, ε = (3.658 A·mL/mg)(303.3 mg/mol) = 1109 A·L/mol. This value is the empirically derived molar absorptivity of cocaine; however, 3.658 A·mL/mg may be used to calculate percent purity since the molecular weight will cancel.

4b. 0.0300 g cocaine HCl sample diluted to 50.00 mL in 0.05 N HCl solution gives 0.600 mg/mL total.

4c. Sample absorbs 0.905 A at 275.4 nm.

4d. Sample c = A/a = 0.905 A/3.658 A·mL/mg = 0.247 mg/mL.

$c_{cocaine}/c_{sample}$ x 100 = (0.247 mg/mL)/(0.600 mg/mL) x 100 = 41.2% cocaine HCl in the sample.

Quantitation Calculation Examples Using Specific Drugs (continued):

5a. **Multiple-drug quantitation** using empirically-derived molar absorptivities for heroin: Sample is known to contain heroin and quinine. No other absorbing or interfering species are present.

5b. $A_T = A_{heroin} + A_{quinine} = a_{heroin}bc_{heroin} + a_{quinine}bc_{quinine} = \varepsilon_{heroin}c_{heroin} + \varepsilon_{quinine}c_{quinine}$ at each λ.

5c. Using **option 2 (serial dilutions)** to make standard solutions:

heroin diluted to 100 mL solution	heroin HCL solution (mg/mL)	A at 278. 8 nm	A at 348.0 nm
0.0000	0.000	0.0000	0.0000
50.0 mL of 0.200 mg/mL	0.100	0.4513	0.0000
50.0 mL of 0.400 mg/mL	0.200	0.9026	0.0000
80.0 mL of 0.500 mg/mL	0.400	1.8050	0.0000
0.0552 g heroin base	0.500	2.2508	0.0000

quinine HCl diluted to 100 mL solution	quinine HCL solution (mg/mL)	A at 278. 8 nm	A at 348.0 nm
0.0000	0.000	0.0000	0.0000
50.0 mL of 0.200 mg/mL	0.100	0.0130	0.1011
50.0 mL of 0.400 mg/mL	0.200	0.0260	0.2028
80.0 mL of 0.500 mg/mL	0.400	0.0500	0.4050
0.0500 g	0.500	0.0680	0.5074

heroin: $slope_{\lambda 278.8}$ = (1.128 A)/(0.250 mg/mL) = 4.513 A·mL/mg
$slope_{\lambda 348.0}$ = (0.0 A)/(0.0 mg/mL) = 0 A·mL/mg

quinine: $slope_{\lambda 278.8}$ = (0.0325A)/(0.250 mg/mL) = 0.130 A·mL/mg
$slope_{\lambda 348.0}$ = (0.253 A)/(0.250 mg/mL) = 1.012 A·mL/mg

Quantitation Calculation Examples Using Specific Drugs (continued):

5d. 0.0500g heroin HCl/quinine HCl sample diluted to 50.00 mL in 0.05 N HCl solution is 1.00 mg/mL total.

5e. Sample absorbs 1.04 A at 278.8 nm and 0.437 A at 348.0 nm

5f. $A' = \varepsilon'_1 c_1 + \varepsilon'_2 c_2$ at λ', $A'' = \varepsilon''_1 c_1 + \varepsilon''_2 c_2$ at λ''

$A_{278.8}$ = (4.513 A·mL/mg)c_h + (0.130 A·mL/mg)c_q
$A_{348.0}$ = (0 A·mL/mg)c_h + (1.012 A·mL/mg)c_q

1.04 $A_{278.8}$ = (4.513 A·mL/mg)c_h + (0.130 A·mL/mg)c_q
0.437 $A_{348.0}$ = 0 + (1.012 A·mL/mg)c_q

$$c_q = \frac{1.04 \ A_{278.8} - (4.513 \ A_h \bullet mL/mg) c_h}{0.130 \ A_q \bullet mL/mg}$$

$$= \frac{1.04 \ A_{278.8} \bullet mg}{0.130 \ A_q \bullet mL} - \left(\frac{4.513 \ A_h \bullet mL \bullet mg}{0.130 \ A_q \bullet mL \bullet mg} \right) c_h$$

$$= 8.00 \ mg/mL - 34.715 \ c_h$$

since $c_q = c_q$, then 8.00 mg/mL - 34.715 c_h = 0.4318 mg/mL

$$c_h = \frac{8.00 \ mg/mL - 0.4318 \ mg/mL}{34.715}$$

$$= \frac{7.568 \ mg/mL}{34.715}$$

$$= 0.2180 \ mg/mL$$

$$c_q = \frac{0.437 \ A_{348.0} - 0}{1.012 \ A_q \bullet mL/mg}$$

$$= 0.4318 \ mg/mL$$

34.715 c_h = 8.00 mg/mL - 0.4318 mg/mL

Quantitation Calculation Examples Using Specific Drugs (continued):

The sample contains 0.2180 mg/mL heroin HCl and 0.4318 mg/mL quinine HCl, or consists of 21.8% heroin HCL and 43.2% quinine HCl.

$$\frac{0.2180\ mg/mL\ heroin}{1.00\ mg/mL\ sample}\ x\ 100 = 21.8\%\ heroin\ HCl\ /\ sample$$

$$\frac{0.4318\ mg/mL\ quinine}{1.00\ mg/mL\ sample}\ x\ 100 = 43.2\ \%\ quinine\ HCl\ /\ sample$$

Safety Concerns:

Eye protection, appropriate gloves, and a laboratory coat should be worn when making the reagent, as concentrated hydrochloric acid is highly corrosive.

Other:

1. *Normal parameter settings include:
   absorbance mode | medium scan speed | 20 nm/cm wavelength scale
   sequential scan mode | 0 - 1, 2, or 3 A range | 350 - 210 nm wavelength range
   0.5 nm slit width

2. Reagents other than 0.05 N HCl may be used for special analyses (although most literature refer

3. Molar absorptivities (ε) are available in literature sources and can be used only for approximating quantitation value. This is useful when calibration standards (e.g.: LSD) are not available. Skoog recommends against using literature values or using a single standard value in quantitation because Beer's law cannot be assumed to be met (see references).

4. Calculations may be done using other units (e.g.: g/L rather than mol/L) as long as consistency is

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