Title: **Determination of Nicotine in Tobacco and Tobacco Products by GC Analysis**

Quality System Compliance Review and Approval

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Release / Revision Record for SOP

Status (Initial/Revision /Retired)	Document Revision Number	Issue/Revision Date	Revision Identification	Revision Author
Initial Release	1	08/08/2014	Original Issue – PPI converted to SOP.	Tammy Blake
Revision	2	02/21/2017	Updated the Scope to include pipe tobacco and cigar filler. Revised the Chemicals and Reagents, and the Procedure sections. Administrative changes were also made.	Beth Hammer
Revision	3	03/27/2017	Corrected the Recovery Standard acceptance criteria.	Beth Hammer
Revision	4	07/14/2017	Changed monitors from CRP1 and CRP2 to CRP1.1 and CRP 2.1	Beth Hammer

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A. SCOPE

1. This procedure describes a process for the gas chromatographic determination of nicotine in cigarette filler, pipe tobacco, cigar filler, and smokeless tobacco (b) (4), MST, snus, dry snuff, and chewing tobacco) using methyl t-butyl ether (MTBE) extraction solution containing quinoline as an internal standard.
2. Samples designated by the customer will require standards addition assay.
3. The method is applicable to tobacco samples whose particle size has been reduced to pass through a 4 mm screen. The sample nicotine range covered by this procedure is 3 to 60 mg/gram (0.3 to 6 weight percent) and results are reported to two decimal places.
4. This method is based on the Centers for Disease Control and Prevention (CDC) protocol.
5. Uncertainty information is found in SOP 095-0061 "SOP: Generalized Procedure for Determining Uncertainty."

B. DEFINITIONS

1. Standard Addition Assay - defined CDC process of preparation of calibration standard in the tobacco matrix to determine if the analysis is impacted by the sample matrix.
2. Internal Quality Control Samples (IQCS) - low concentration and high concentration:
    a. CORESTA Reference Product 1 (such as CRP1.1, IQCS Low) -Swedish-style Snus smokeless tobacco monitor product, without any flavorings added, packaged in a plastic can containing ~ 24 - 1g pouches that can be obtained from the Tobacco Analytical Service Laboratory (TASL) at North Carolina State University.
    b. CORESTA Reference Product 2 (such as CRP2.1, IQCS High) - American-style loose moist snuff smokeless tobacco monitor product packaged in a plastic cans containing 34 grams that can be obtained from the Tobacco Analytical Service Laboratory (TASL) at North Carolina State University.

C. RESPONSIBILITIES

1. The designated trained analyst performing the method is responsible for following all steps of the procedure and documenting and reporting any procedural deviations from the method to lab management.
2. Personnel using this test method are responsible for conducting the analysis in a manner consistent with the safety policies of ALCS.

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D. VALIDATION

1. This procedure is based on "A Uniform Protocol for the Analysis of Nicotine, Total Moisture, and pH in Smokeless Tobacco Products" from CDC published in the 2009 Federal Register.
2. Validation is not required for analysis of products within the intended scope of the CDC protocol (e.g., snus, moist snuff, etc.) However, the laboratory must demonstrate performance by documenting:
   a. tests performed by trained personnel;
   b. a monitored quality control program;
   c. inter-lab studies with demonstrated proficiency; and
   d. audits of the procedure.
3. Tobacco products or product configurations not explicitly defined in the CDC protocol but capable of being prepared as described in the document can be analyzed using the CDC protocol (i.e., ground to a particle size of < 4 mm.) The laboratory must demonstrate performance as described previously.
4. (b) (4)

Grand Average	Standard Deviation	% RSD
(b) (4)		

E. EQUIPMENT AND APPARATUS

1. Equipment and Apparatus Required
   a. Gas Chromatograph:
      1) Agilent Model 6890, with split/splitless capillary injector capability, flame ionization detector, and ChemStation software, or equivalent.
      2) Capillary column: HP-5, cross-linked 5% phenyl-methylpolysiloxane, 30 m length x 0.32 or 0.25 mm ID, and a film thickness of 0.25 µm, Agilent, or equivalent.
      3) Syringes: 10-µL for auto-injector, PTFE tipped, or equivalent.
      4) Septa: 11-mm, any manufacturer.
      5) Inlet liner: Deactivated glass with glass wool, Agilent, or equivalent.
      6) O-ring for inlet liner: Agilent, or equivalent.
   b. Orbital shaker, New Brunswick Scientific Co., Edison NJ, or equivalent, used for sample extraction, capable of 200 - 275 rpm.

c. Glassware:

1) Amber glass bottles, 60mL, and 120mL size, with Teflon lined caps.
2) Disposable plastic, or glass Pasteur pipettes.
3) Extraction vial, 60-mL, amber, I-Chem vials with Teflon lined caps, I-Chem, or equivalent.
4) GC vials, 1.5-mL, amber, Fisher #03-391-6 or equivalent.
5) GC vial caps, 11-mm crimp seal, Thermo # C4011-2A, or equivalent
6) Pipettes, volumetric, Class A, various sizes.
7) Gas-tight syringes, 250-µL, 500-µL, 2.5-mL and 5-mL, Hamilton # 81100, 81217, 81417, 81517, or equivalent.
8) Volumetric flasks, Class A, 50-mL, 100-mL, 250-mL, 1000-ml, and 2000-mL with ground glass stoppers.
9) Graduated Cylinder, Certified, 10-mL, Certified Class A, To Contain, e.g. Fisher, or equivalent.
10) Graduated Cylinder, Certified, 50-mL, Certified Class A, To Contain, e.g. Fisher, or equivalent.
11) 3mm glass beads, Pyrex #72683, or equivalent.
12) Erlenmeyer flasks with caps, 125mL, Fisher # 11-888 and 11-8881.

d. Pipettes:

1) Repeater pipette tips, 5 mL, e.g. Eppendorf Combitips plus Catalog #22 26 640-3, Fisher Scientific.
2) Repeater Plus, e.g. Catalog #21-380-9, Fisher Scientific.

e. Rotary extractor capable of holding test tube racks used to extract samples by repeated inversion of the sample tubes. (Capable of running between 13-17 rpm's.)

f. Volumetric Dispensers:

1) 1-10mL adjustable, e.g. Fisher # 13-642-891, or equivalent.
2) 5-50mL adjustable, e.g. Fisher # 13-642-889, or equivalent.

2. Instrument Setup

a. Set-up and operate the gas chromatograph and autosampler according to the manufacturer's instructions.

b. Typical GC operating conditions:

Parameter	Set Point
Carrier gas	Helium
Injection temperature	250°C
Injection mode	Split (40:1)
Injection volume	1.0 µL
Constant flow rate	1.7 mL/min
Initial temperature	110°C
Initial hold time	0 min
Temperature ramp A	10 °C/min
Final temperature A	185°C
Temperature ramp B	25°C
Final temperature B	245°C
Final hold time B	2 min
Detector	250°C
Total run time	~ 12 min

c. Typical Agilent 6890 GC integration parameters for nicotine analysis are listed below. The integration set points listed may be modified as necessary to improve sensitivity. Any modifications to these settings must be recorded in the instrument logbook.

Time	Parameter	Set Point
Initial	Slope Sensitivity	10
Initial	Peak Width	0.02
Initial	Area Reject	1
Initial	Height Reject	1
Initial	Shoulders	OFF
0.100 min	Baseline at Valleys	ON

3. Instrument Maintenance

Note: Prior to performing a calibration any necessary routine maintenance should be performed. Document all maintenance in the instrument logbook.

a. The GC septum should be changed as necessary.
b. The inlet liner should typically be changed every 100 injections or as necessary depending upon sample loads and sample matrix.
c. Replace GC syringes as needed.

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F. CHEMICALS AND REAGENTS

1. Chemicals Required

   a. Gases:

      1) Air: filtered, house compressed air
      2) Helium: Grade 5, 99.999 % purity
      3) Hydrogen: hydrogen generator or Ultra High Purity (UHP) Grade 5, 99.999 % purity

   b. Reagents:

      1) Methyl t-butyl ether (MTBE), HPLC Grade, CAS# 1634-04-4, e.g. Fisher # E127-4 or equivalent.
      2) ISO Guide 34 Nicotine stock solution, 10mg/mL in methanol with 0.4mg/mL quinolone internal standard, CAS # 54-11-5, minimum 99% purity, SPEXCertiPrep, Inc, Catalog # VO-FSPA-108-50. Nicotine must be stored in a freezer. See COA for details.
      3) ISO Guide 34 Nicotine calibration check standard stock solution, 5mg/mL in methanol with 0.4mg/mL quinolone internal standard, CAS # 54-11-5, minimum 99% purity, SPEXCertiPrep, Inc, Catalog # VO-FSPA-109-15. Nicotine must be stored in a freezer. See COA for details.
      4) Quinoline internal standard stock solution, 40mg/mL in methanol, CAS# 91-22-5, >98% purity, SPEXCertiPrep, Inc, Catalog # VO-FSPA-107-110.

         Note: All Stock solutions must be stored in the freezer at -20 ± 5°C

      5) Sodium hydroxide (NaOH) Solution, for example, 2N sodium hydroxide (NaOH) solution CAS# 1310-73-2, Fisher # 21 -380-9 or equivalent.

2. Reagent Preparation

   Note: See COA for the nicotine stock solution expiration date. All working standards have the same expiration as the stock solutions. Equivalency testing is required every three months or when new standards are purchased and put into service.

   Note: The preparation of standards and/or reagents may be scaled up or down to meet laboratory needs. If scaling is performed, special care must be used to ensure that accurate concentrations are obtained.

   a. 2N Sodium Hydroxide (NaOH) Solution

      1) Purchase 2N Sodium hydroxide (NaOH) solution or,

2) Prepare 2N NaOH by diluting from a higher concentration of NaOH with milli Q H2O. Calculation is as follows:

$$(N \times V)_{initial} = (N \times V)_{final}$$

e.g., using 5N NaOH solution:

$$5N \times V_{initial} = 2N \times 1000$$

$$V_{initial} = 2000/5 = 400$$

Use 400 mL of 5N NaOH then add to volume of 1000 mL with H_2O to make a 1-liter batch of 2N NaOH solution.

b. Internal Standard Stock Solution (40 mg/mL) used for the preparation of Extraction Solution.

1) Extraction Solution (prepared to contain 0.4 mg/mL Quinoline Internal Standard). To prepare 6L, pipette 60 mL of the internal standard stock solution, quinoline 40 mg/mL, into a 2-liter volumetric flask and dilute to volume with MTBE. Transfer solution to a carboy and add 4 more liters of MTBE. Volume can be scaled as needed.

a) Verify that the quinoline internal standard GC peak area of the extraction solution of is equivalent to the quinoline internal standard GC peak area in the working standards by:

(1) Running the current nicotine calibration standards and six replicates of the newly prepared extraction solution on the GC.
(2) Averaging the internal standard peak area for the calibration standards and the extraction solution replicates.
(3) Ensuring that the difference between the two is within the allowable target limit of 5 percent and document in the Nicotine Standards Logbook.

(a) Label the carboy with the contents and expiration date. The expiration date of the Extraction Solution shall not exceed the expiration date of the Quinoline, 40 mg/mL, stock solution located on the COA.

3. Standard Preparation

   a. Nicotine Stock solution (10 mg/mL nominal): Per the table below, pipette the exact amount of the Nicotine Stock Solution and Nicotine Calibration Check Standard Stock Solution into each flask. 0.06, 0.1, 0.2, 0.4, 0.8, and 1.2 mg/mL, and nicotine standards and 0.6 mg/mL CCS:

Standard Number	Nicotine Stock Solution (mL)	Flask Volume (mL)	Nominal Nicotine Concentration (mg/mL)	Nominal Nicotine Concentration (weight %)
1	0.3	50	0.0600	0.300
2	0.5	50	0.10	0.50
3	1	50	0.20	1.00
4	20	500	0.40	2.00
5	4	50	0.80	4.00
6	6	50	1.20	6.00
Standard Number	Nicotine CCS Stock (mL)	Flask Volume (mL)	Nominal Nicotine Concentration (mg/mL)	Nominal Nicotine Concentration (weight %)
CCS	12	100	0.6	0.4

      1) Dilute to mark with extraction solution and mix.
      2) Transfer to amber bottles labeled with calculated, actual nicotine concentration to 2 decimal places.

   b. 0.4 mg/mL nicotine standards

      1) Transfer a portion to amber bottles labeled with calculated, actual nicotine concentration to 2 decimal places.
      2) Transfer the balance to an amber bottle labeled as "Nicotine Recovery Standard" with the calculated concentration to 2 decimal places.

   c. Calibration Check Standard (CCS) (0.6 mg/mL nicotine).

      1) Into a 100 mL volumetric flask, pipette 12 mL of the Nicotine Check Standard Stock Solution, 5 mg/mL, as described in the table above.
      2) Dilute to mark with extraction solution and mix.
      3) Transfer a portion to amber bottles labeled as the Calibration Check Standard (CCS) with the calculated, actual nicotine concentration to 2 decimal places.

      Store all working standards in a refrigerator at 4°C ± 3°C.

d. Standards Verification

1) Use the Nicotine Filler Standards Verification form to verify new standards preparation.

2) Management shall review the verification form and authorize use of new standards.

G. SAMPLE REQUIREMENTS

1. Care should be taken in handling the samples stored in the freezer / refrigerator. Sufficient time should be given to allow the sample material to reach room temperature before sample preparation.
2. The target tobacco weight for a replicate analysis of loose tobacco product is 1.000 ± 0.020 grams.
   a. Tobacco: tobacco larger than 4mm shall be ground at ambient temperature as described in WI 097-1108 "Sample Preparation.
   b. Cigarette filler: shall be ground at ambient temperature as described in WI 097-1108 "Sample Preparation.
   c. Cigar filler: cigar filler shall be freeze ground as described in WI 097-1108 "Sample Preparation".
   d. Pipe Tobacco: pipe tobacco shall be freeze ground as described in WI 097-1108 "Sample Preparation".
   e. Pouched smokeless tobacco products for CDC reporting: Open a sufficient number of pouches and discard the paper. Thoroughly mix the tobacco for analysis.
   f. Pouched smokeless tobacco products for FDA reporting: Unit pouches shall be analyzed and include both the paper and tobacco. Cut the pouches in half and add the tobacco and paper directly into the extraction vessel.
   g (b) (4) for CDC and FDA reporting: the tobacco shall be removed from the (b) (4) and ground as described in WI 097-1108.

H. PROCEDURE

1. Sample Handling

Note: Perform a volume check on the dispensers using a certified graduated cylinder prior to use and record results on the Nicotine Filler Extraction Solution Dispensing Volume Check form and the Nicotine Filler Sodium Hydroxide Dispensing Volume Check form.

a. Monitor (IQCS) Preparation

1) In tandem with preparation of the first sample of a GC run, prepare the Low and High Internal Quality Control Samples (IQCS).

a) Prepare an appropriate number of Low IQCS by weighing into the extraction vial a pre-determined number of opened CRP1.1 pouched products (normally 2) that has been allowed to come to room temperature. Record the exact weight, including the tobacco plus pouch.
b) Prepare an appropriate number of High IQCS by weighing into the extraction vial 1.000 ± 0.020 grams of CRP2.1 that has been allowed to come to room temperature. Record the exact weight.

b. Sample Preparation

Note: Typically CDC Samples are prepared and run in duplicate, and FDA samples require 7 replicates.

1) Loose tobacco: Weigh 1.000 ± 0.020 grams of sample into a labeled extraction vial and record the exact weight to the nearest 0.0001g.
2) (b) (4) Weigh 1.000 ± 0.020 grams of sample into a labeled Erlenmeyer flask. Record the exact weight to the nearest 0.0001g. Add 5-10 glass beads to the extraction flask
3) Pouched smokeless tobacco for FDA reporting: separate the tobacco from the paper and carefully place the tobacco into the extraction vessel along with the pouch paper. Use as many whole portion products (tobacco + pouch material) as necessary to achieve a target weight between **0.8 – 1.5 grams**. Record the exact weight to the nearest 0.0001g.
4) For samples requiring Standard Addition, weigh an additional 1.000 ± 0.020 grams of sample into each of five (5) extraction vials. Record the exact weight to the nearest 0.0001g. For FDA reporting of pouched products, add a sufficient number of pouches, as discussed above (tobacco separated from the paper), to reach a target weight of 0.8 – 1.5 g and record the exact weight to the nearest 0.0001g. Using a gas-tight syringe or a mechanical pipette, add 0.5, 1, 2, 3, and 4 mL, respectively, of the Nicotine Stock Solution (10mg/mL) into the five extraction vials and cap. The average value of the unfortified samples prepare in 1) immediately above will be used as the standard addition blank.

Note 1: For this method, the standard addition preparations generate a set of five (5) matrix-matched standards whose calibration curve is used to quantitate the two (2) unfortified samples (7 replicates total). It is used for samples where there are constituents that elute at the same retention time as the quinoline internal standard peak; thereby causing inaccurate quantitation. Spearmint-flavored tobacco products are known to require standard addition.

The need for standard addition is indicated by an elevated area count for quinolone compared to other samples and standards in the batch.

Note 2: For FDA samples requiring standards addition, a total of 7 unfortified samples must be prepared and quantitated against the 5 fortified samples that are used to construct the standard addition curve. This will result in 12 replicates total.

5) Extraction of Monitors (IQCSs), Samples and Standard Addition preparations

Note: After rotating, allow the solvent and nicotine supplemented samples and the blank to separate (maximum 2 hours). In the event that samples cannot be analyzed immediately upon extraction, extracts may be vialed and stored in a refrigerator, 4°C ± 3°C, for up to four days.

a) Add 5 mL of 2N NaOH (use 10 mL if analyzing (b) (4) products) into the extraction vials containing IQCS's and samples.
b) Swirl to wet the sample and equilibrate for a minimum of 15 minutes.
c) Pipette 50.0 mL of extraction solution into each vial, cap and tighten.
d) Place the extraction vial on the rotator. Use an orbital shaker if analyzing (b) (4) products. Rotate or shake for a minimum of two hours. Record the time.
e) Transfer an aliquot of the organic layer into a GC vial and cap.

c. Nicotine Recovery Check (Checks 1-3), in triplicate:

1) Pipette 0.5 mL of 2N NaOH into an extraction vial.
2) Pipette 5 mL of the 0.4 mg/mL nicotine standard.
3) Shake the contents vigorously and allow the phases to separate.
4) Transfer an aliquot of the top organic phase to a GC vial.

d. Nicotine Recovery Check for Standards Addition (Checks 4-6) , in triplicate (this is prepared only for batches that contain one or more standard addition samples)

1) Pipette 0.5 mL of 2N NaOH into an extraction vial.
2) Pipette 5 mL of Extraction Solution.
3) Pipette 5 mL of the 0.4 mg/mL nicotine recovery standard.
4) Shake the contents vigorously and allow the phases to separate.
5) Transfer an aliquot of the organic phase to a GC vial and cap.

2. Calibration

   a. A new calibration curve should be acquired daily. Fresh aliquots of Nicotine working standards, Cal 1 through Cal 6, must be used. In order to conserve the working standards, a low-volume autosampler vial or glass autosampler vial insert may be used (conical or straight walled, 200 - 400 µL volume, filled half way).

3. Analysis

   Load the GC autosampler typically as follows:

   1) A vial of extraction solution blank.
   2) The six vials containing the working standards (Cal 1 – Cal 6) in increasing order of concentration.
   3) A second vial of extraction solution blank.
   4) Nicotine Recovery Checks solutions (Checks 1-3)
   5) Nicotine Recovery Checks for Standard Addition solutions (Checks 4 -6), if needed
   6) CCS
   7) The first set of prepared Low and High IQCS's.
   8) The Sample solutions.
   9) The second set of Low and High IQCS's.
   10) End batch with a CCS.

   Note: There is not a requirement for the number of times the CCSs are analyzed in a batch except that all samples must be bracketed by passing CCSs. The analyst should determine if it is appropriate to add CCSs in the middle of a sample batch based upon batch size and the length of a run.

4. Calculations and Reporting

   a. Determination of actual nicotine concentration of Calibration Standards:

$$\text{Nicotine (mg/mL)} = \frac{\text{Actual Nicotine Stock Conc. (mg/mL)} \times \text{milliliters of Stock added}}{\text{Volume of Flask (mL)}}$$

   b. Linear regression calculation:

$$y = a + bX$$

   Where:

   X = Concentration of nicotine in mg
   y = Response of Nicotine (see below)
   a = Intercept of the ordinate (y-axis)
   b = Slope of the curve

1) For new standard verification: y = Peak area $_{nicotine}$
2) For nicotine calibration standards: y = Area ratio = (Peak area $_{nicotine}$/Peak area $_{quinoline}$).
3) For standard addition recovery: y = (Nicotine area ratio – Standard blank area ratio).
4) For standard addition analysis: y = (Nicotine area ratio – Standard blank area ratio).
5) The sample nicotine concentration is calculated from the following rearranged linear regression equation:

$$X = \frac{(Y - a)}{b}$$

c. The following equations are used in the CDC Nicotine Calculation Worksheet for samples not requiring standard addition

1) Nicotine Recovery:

$$\text{Nicotine Recovery} = \frac{\text{Nicotine}_{\text{Calculated}}}{\text{Nicotine}_{\text{Actual}}}$$

2) Calculation of sample nicotine concentration:

$$\text{Nicotine (mg/g)} = \frac{(\text{Nicotine conc. per calibration stds. (mg/mL)} \times \text{Vol. Extraction Soln. (mL)})}{(\text{Sample Wt. (g)} \times \text{Recovery}_{\text{Tobacco Matrix}})}$$

d. The following equations are used in the CDC Nicotine Calculation Worksheet for Standard Addition Analysis:

1) Nicotine Recovery:

$$\text{Nicotine Recovery} = \frac{\text{Nicotine}_{\text{Calculated}}}{\text{Nicotine}_{\text{Actual}}}$$

2) Nicotine Recovery Comparison:

$$\text{Recovery Comparison (\%)} = \frac{\text{Recovery}_{\text{Aqueous Matrix}} - \text{Recovery}_{\text{Tobacco Matrix}}}{\text{Recovery}_{\text{Aqueous Matrix}}} \times 100$$

3) Calculation of sample nicotine concentration:

If recovery comparison difference is less than 10%:

$$\text{Nicotine (mg/g)} = \frac{(\text{Nicotine conc. per calibration stds. (mg/mL)} \times \text{Vol. Extraction Soln. (mL)})}{(\text{Sample Wt. (g)} \times \text{Recovery}_{\text{Tobacco Matrix}})}$$

If recovery comparison is equal to or more than 10%:

$$\text{Nicotine (mg/g)} = \frac{\text{Nicotine conc. per standard addition assay (mg/mL)} \times \text{Vol. Extraction Soln. (mL)}}{\text{Sample Wt. (g)}}$$

e. Calculation of Free Nicotine

For $B + H^+ \rightleftharpoons BH^+$ $\quad pH = pKa + \log\frac{[B]}{[BH^+]}$

$$\%\text{ unionized (free) nicotine} = \frac{\frac{[B]}{[BH^+]}}{\frac{[B]}{[BH^+]}+1} \times 100$$

Where: [B] = amount of unionized (free) nicotine

$[BH^+]$ = amount of ionized nicotine

Note: pKa of Nicotine = 8.02

$$\text{Total Free Nicotine (mg/g)} = \text{Total Nicotine} \times \frac{\%\text{ unionized (free) nicotine}}{100}$$

f. Calculations for standard additions, recoveries, and sample results are performed in the CDC Nicotine Calculation Worksheet or in MP-LIMS. If not calculated in MP-LIMS, CDC Nicotine calculations are cross-verified by an authorized analyst.

g. Determination of the total difference (D) between IQCS run means and run chart means in standard deviation (S) units.

$$D(\text{standard deviations}) = \left|\frac{\bar{X}_{\text{low IQCS run}} - \bar{\bar{X}}_{\text{low IQCS chart}}}{S_{\text{low IQCS chart}}}\right| + \left|\frac{\bar{X}_{\text{high IQCS run}} - \bar{\bar{X}}_{\text{high IQCS chart}}}{S_{\text{high IQCS chart}}}\right|$$

$$S_{\text{low or high IQCS chart}} = \frac{\text{Upper 2S limit} - \bar{\bar{X}}_{\text{IQCS Chart}}}{2}$$

Note: “Run” means “sample set.”

5. Quality Control and Acceptance Criteria
   a. When the sequence is complete print all chromatograms, batch report, and the calibration curve.
      1) Check the report to assure that all standards were analyzed, and that there were no obvious instrument malfunctions.
      2) If any problems are found (i.e. missing data, incorrect bar code numbers, etc.) correct the problem(s) and reanalyze the samples or reprocess the data.
      3) Check the data to assure that the extracting solution amount and weights are correct and that multipliers, if appropriate, are correct.
      4) Evaluate the coefficient of determination (R^2) for the calibration standards. The R^2-value for the calibration must be greater than 0.990 for the calibration to be acceptable.
      5) Calibration Check Standards (CCSs): The validity of the calibration curve is checked after calibration and throughout an analytical batch by injecting the CCS. All samples must be bracketed by passing CCSs. The %RCR for the CCS should be within ±15% of the nominal nicotine concentration. A CCS result that does not pass the acceptance criteria suggests that the calibration curve is no longer valid. The samples analyzed after the last passing CCS must be reanalyzed with a new calibration unless there is an assignable cause and management approval.
      6) Blank (Extraction Solution): The blank injection is used to check for contamination and any carryover originating from sample preparation or the analytical procedure. If the calculated concentration of nicotine in the blank exceeds half of the concentration of Cal 1, the entire batch should be considered suspect and appropriate corrective measures should be taken.
      7) Check the retention times. Assure that the retention time of the internal standard (quinoline) peak and the nicotine retention time are consistent throughout the gas chromatographic sequence run. The retention times for the internal standard and nicotine should not vary by more than ± 0.50 minutes from the retention times listed for each component in the calibration table. Inconsistency in the retention times could be a source of potential problems and should be investigated.
      8) Internal Standard Area Counts: Specific flavor ingredients are known to coelute with the internal standard and cause inaccurate quantitation. The raw internal standard area count of each sample must be compared to the

average raw internal standard area count of the calibration standards. If the sample internal standard area counts differ from the average calibration standard internal standard area counts by more than 10%, the sample must be reanalyzed by the standard addition method.

9) Monitors (IQCSs): Plot the current mean nicotine concentrations for the Low and High IQCS's on their respective Quality Control charts. The rules and information on how to address out-of-control conditions are documented on the control charts.
10) Recovery Standards: The triplicate measurements of the External Standard (and Standard Addition Recovery solutions should have Nicotine Recovery values ≥ 0.980.

I. REFERENCES

1. Department of Health and Human Services, Centers for Disease Control and Prevention, Revised Protocol for Analysis of Nicotine, Total Moisture, and pH in Smokeless Tobacco Products, Federal Register / Vol. 74, No. 4 / Wednesday, January 7, 2009 / Notices, pages 712 - 719.
2. SOP 095-0061Generalized Procedure for Determining Uncertainty
3. WI 097-1108 Sample Preparation by Lancaster

J. FORMS

1. 099-5105 Nic Filler Standards Validation and CCS-CDC
2. 099-5101 CDC Nicotine Calculation Worksheet
3. 099-5102 Regulatory Checklist
4. 099-5107 Nicotine Filler Sodium Hydroxide Dispensing Volume Check Log
5. 099-5106 Nicotine Filler Extraction Solution Dispensing Volume Check Log
6. 099-5103 CDC-NIC Monitor Results