Title: Determination of Flavors in Smokeless Tobacco by GC-MS	Control #: ST-TM-410-634	Revision #: 1.0
	Approval Date: 09/22/2015	Effective Date: 10/1/2015
Test Method Owner: ALCS / RD&E / Analytical Technical Services		

Table of Contents

Title:	Control #:	Revision #:
Determination of Flavors in Smokeless Tobacco by GC-MS	**ST-TM-410-634**	**1.0**
	Approval Date:	**Effective Date:**
	09/22/2015	**10/1/2015**
Test Method Owner: ALCS / RD&E / Analytical Technical Services		

NOTICE
This method may involve the use of hazardous substances and/or equipment. The user must not assume that all of the safety issues associated with its use have been described. Prior to use of this method, the user is responsible for establishing appropriate safety and health practices and determining the applicability of regulatory requirements.
The employee performing this method must be trained according to the safety guidelines specific to the job task and area of assignment. The employee must use all appropriate safety equipment referenced by the facility's safety guidelines. Copies of Safety Data Sheets (SDS) are available from the Altria Safety Health and Environmental intranet site, facility safety department or the area supervisor.

A. Scope

1. This test method describes the procedure for the quantitative analysis of selected flavor compounds in finished goods and in-process tobacco materials by Gas Chromatography-Mass Spectrometry (GC-MS).

B. Definitions

1. Finished Goods - Product that has been packaged and is ready to be introduced into the commercial market.

C. Responsibilities

1. Laboratory management shall ensure that personnel performing this method have demonstrated competence and documented proficiency.
2. Laboratory personnel are responsible for performing testing and documenting information as defined in this method. Any significant deviations from this method are to be documented and reported to laboratory management.

D. Equipment and Apparatus

1. Equipment and Apparatus Required
   a. Agilent GC-MS system, or equivalent, with the following accessories:
      1) Autosampler.
      2) Split/Splitless injection port with Electronic Pressure Control.
      3) MSD Ion source with EI capability.
      4) Agilent GC-MS Chemstation data system, capable of acquiring, processing and archiving data.
   b. Analytical column: Restek 5Sil MS with Integra-guard. 30m x 0.25mm x 0.25mm.
   c. Glass vials (20 mL minimum) with Teflon lined septa.
   d. 2 mL Autosampler vials for final extract.

e. Various sized amber vials to store calibration solutions.

f. Vortexer.

g. Various syringes, Eppendorf pipets or other small volume measuring devices.

h. Volumetric Flasks, Class “A”.

i. Glass pipets.

j. Metal or Teflon spatulas.

k. Analytical balance capable of weighing to 0.001g.

2. Instrument Setup

a. GC-MS Conditions

Inlet Pressure	Constant at ~11 psi
Inlet Temp	250°C
Split Ratio	20:1
Oven Program	
Initial Temp	75°C
Hold Time 1	0.1 min
Ramp Rate 1	20°C/min
Final Temp 1	175°C
Hold Time 2	0 min
Ramp Rate 2	35°C/min
Final Temp 2	270°C
Hold Time 3	1.5 min
Total Run Time	~9.3 min
Transfer Line	280°C
Source Temp	230°C

3. Instrument Maintenance

a. Periodic verification of the peak shapes and the ability to generate a linear curve are indicators of whether the system requires maintenance.

1) The Inlet Liner and gold seal should be replaced and the front of the column clipped if the component peaks of interest are tailing.

2) A loss in sensitivity and/or poor instrument tuning (i.e. split peaks) is an indicator that the Mass Spectrometer source requires cleaning.

b. Annual preventive maintenance should be performed on the instrument to ensure continued good working condition.

E. Chemicals and Reagents

1. Chemicals Required

a. Ethanol, Reagent grade

b. 6-methylcoumarin 99.9% purity, Aldrich P/N M36203-100G

c. Flavor Stock Standard, Custom Flavor Mix prepared in Ethanol, Restek P/N #568337 or equivalent, with appropriate Certificate of Analysis.

2. Reagent Preparation

a. Ethanol Extraction Solution

1) Weigh 6-methylcoumarin to a final known concentration in Ethanol in a volumetric flask.

a) Example: Weigh 1.0g 6-methylcoumarin in a 1000 mL volumetric flask and fill the flask to volume with Ethanol.

2) Depending on the volume of samples to be tested, the volume of extraction solution may vary.

3) The amount of the internal standard in the extraction solution will be documented in the quantitation software. Chemstation allows for the extraction solution to be a different concentration than the concentration in the calibration standards. This information is captured in the detailed compound information of the calibration table.

3. Standard Preparation

a. Internal Standard Stock Solution (~1 mg/mL 6-methylcoumarin)

1) Weigh 0.1g of 6-methylcoumarin into a 100 mL volumetric and fill the flask to volume with Ethanol.

NOTE: 6-methylcoumarin is used as the internal standard and will be added at the same volume to each standard to be equivalent to the final concentration in the sample extracts.

b. Flavor Stock Standard

1) The Flavor Stock Standard mix is diluted in Ethanol to the required levels for analysis. Typical concentrations of the flavor components in the Stock Standard are listed below.

Title: Determination of Flavors in Smokeless Tobacco by GC-MS	Control #: ST-TM-410-634	Revision #: 1.0
	Approval Date: 09/22/2015	Effective Date: 10/1/2015
Test Method Owner: ALCS / RD&E / Analytical Technical Services		

Component	Code	Stock Conc. (ppm)
(b) (4)	CHR01	(b) (4)
	WSK02	
	CPN01	
	SPR01	
	PCH02	
	STR01	
	APP02	
	WSK01	
	CTR01	
	BRR02	
	MNT02	
	WGN01	
	CPN04	
	CHR02	
	DRK01	
	RSN01	
	ISTD	

c. Calibration Standards

1) Add the appropriate volume of Flavor Stock Standard Solution (as listed in the table below) to 50 mL volumetric flasks.

Standard #	Flavor Stock (mL)
1	0.025
2	0.1
3	0.25
4	0.5
5	2
6	6

2) Add 0.25 mL of Internal Standard Stock Solution to each flask, and fill each flask to volume with Ethanol.

3) Final concentrations of the components in the Calibration Standards are calculated based on the concentrations in the certified Flavor Stock Standard Solution. Typical concentrations of the flavor components in the Calibration Standards are listed below.

Title: Determination of Flavors in Smokeless Tobacco by GC-MS	Control #: ST-TM-410-634	Revision #: 1.0
	Approval Date: 09/22/2015	Effective Date: 10/1/2015
Test Method Owner: ALCS / RD&E / Analytical Technical Services		

	Calibration Standard Concentration (ppm)					
Component	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
CHR01	(b) (4)					
WSK02						
CPN01						
SPR01						
PCH02						
STR01						
APP02						
WSK01						
CTR01						
BRR02						
MNT02						
WGN01						
CPN04						
CHR02						
DRK01						
RSN01						
ISTD						

4) Standards are prepared and documented using reference document FN64.0002RF GCMS Flavor Calibration Standards. The standard concentrations may be varied as needed by the analyzing lab as long as the low and high standards are within the linear range of the instrument as indicated by the validation.

5) Not all components listed within this document are required to be analyzed each time the method is run. The laboratory may customize the standard mix as needed providing all information is documented and any component not included in the standard mix is not reported.

d. Calibration Check Solution (CCS)

1) Add the appropriate volume of Flavor Stock Standard Solution (as listed in the table below) to 50 mL volumetric flasks.

CCS	Flavor Stock (mL)
Low	(b) (4)
High	

2) Add 0.25 mL of Internal Standard Stock Solution to each flask, and fill each flask to volume with Ethanol.

3) Final concentrations of the components in the Calibration Standards are calculated based on the concentrations in the certified Flavor Stock Standard Solution. Typical concentrations of the flavor components in the Calibration Check Solutions are listed below.

Title: Determination of Flavors in Smokeless Tobacco by GC-MS	Control #: ST-TM-410-634	Revision #: 1.0
	Approval Date: 09/22/2015	Effective Date: 10/1/2015
Test Method Owner: ALCS / RD&E / Analytical Technical Services		

Component	CCS Low (ppm)	CCS High (ppm)
CHR01	(b) (4)	
WSK02		
CPN01		
SPR01		
PCH02		
STR01		
APP02		
WSK01		
CTR01		
BRR02		
MNT02		
WGN01		
CPN04		
CHR02		
DRK01		
RSN01		
ISTD		

F. Sample Requirements

1. Minimum sample required for analysis is 5g, which allows for re-analysis and duplicate analysis if needed.

G. Test Procedure

1. Sample Handling
   a. Samples are to be stored at $\leq$4°C upon receipt if not analyzed within 24 hours of receipt.
   b. Samples should be allowed to come to room temperature prior to analysis.
   c. Samples at the production facility are collected according to USSTC Work Instruction NW63.0065 Collection and Preparation of Finished Goods Samples for Flavor Analysis by GCMS.
2. Sample Preparation
   a. Weigh ~ 0.5g of each sample into a labeled sample extraction container. Record the actual weight.
   b. Add 5 mL of extraction solution to each sample.
   c. Vortex the sample for 10 minutes.
   d. Allow the tobacco to settle after vortexing.
   e. Transfer an aliquot of each sample extract into a labeled autosampler vial.
3. Calibration

a. The components are identified and added to the calibration table in order to generate a calibration curve. Quantitation Ions are used for identification as well as concentration calculations

Component	Code	Quantitation Ion
(b) (4)	CHR01	(b) (4)
	WSK02	
	CPN01	
	SPR01	
	PCH02	
	STR01	
	APP02	
	WSK01	
	CTR01	
	BRR02	
	MNT02	
	WGN01	
	CPN04	
	CHR02	
	DRK01	
	RSN01	
	ISTD	

b. Calibration is performed by injecting each standard using the same analytical parameters for samples.

c. The linearity of the curve can be evaluated using linear regression with a correlation coefficient of 0.990 or better or by average response factors with an average response factor of $\leq$ 20%.

4. Analysis

a. GC-MS Injection sequence:

Blank

Calibration Standards 1- 6 (more levels may be used if needed)

Blank

CCS Low

CCS High

Blank

Samples

Blank

CCS Low

CCS High

Title: Determination of Flavors in Smokeless Tobacco by GC-MS	Control #: ST-TM-410-634	Revision #: 1.0
	Approval Date: 09/22/2015	Effective Date: 10/1/2015
Test Method Owner: ALCS / RD&E / Analytical Technical Services		

NOTE: If different flavors are being analyzed, blank samples should be run between flavor systems to prevent carryover (WG Samples, Blank, Mint Samples, Blank, etc.)

b. Using the Mass Spectrometer software, the components of interest are identified and the concentrations are calculated using the calibration curve stored with the quantitative method.

c. All components are identified and quantitated by retention time and a specific quantitation ion to eliminate contributions from a co-eluting component.

d. All results should be verified by a trained analyst to eliminate false positive and negative reporting.

1) Analysts should evaluate each identified component to ensure that the mass spectra match the known component spectra.

2) Noise in the baseline can affect integration and cause incorrect peaks to be identified as a component of interest.

3) Baseline issues or contributions from coeluting peaks with similar quantitation ions can affect integration causing a higher calculated concentration.

4) Care should be taken to maintain the instrument's injection port (liner and seal) in order to limit the possibility of contamination contributions.

5. Calculations and Reporting

a. The concentration generated by the Chemstation software is the absolute concentration of the injected solution. In order to calculate the actual component concentration the following equation is used:

$$\text{Componet Concentration} = \text{Instrument Concentration} \times \frac{\text{ExtractionSolution(mL)}}{\text{SampleWeight(mg)}} \times \text{DilutionFactor}$$

6. Quality Control and Acceptance Criteria

a. All unknown sample quantitation results must be verified to be within the calibration range. Appropriate dilutions of samples may be done to assure that samples are within the calibration range.

b. Calibration Check Solution (CCS)

1) The CCS is used to verify that the calibration curve is performing as expected at different levels. Ideally a low and a high CCS should be used. If the method is being used as a "pass/fail" for components, the CCS should represent the level of interest for the end user.

2) The CCS results must be compared to the established concentration for each component in the solution.

a) If the method is being used as a quantitative analysis, all components of interest must fall within ±15% of the established component concentrations.

Title: Determination of Flavors in Smokeless Tobacco by GC-MS	Control #: ST-TM-410-634	Revision #: 1.0
	Approval Date: 09/22/2015	Effective Date: 10/1/2015
Test Method Owner: ALCS / RD&E / Analytical Technical Services		

b) If the method is being used as a "pass/fail" for components, all components of interest must fall within ± 30% of the established component concentrations.

c) If a component in the CCS does not meet the QC Criteria:

(1) The solution should be re-vialed and re-injected. A subsequent failure would indicate the need for a new calibration curve or instrument maintenance.

(2) If the failing component in the CCS is not a component of the samples in the analysis run, the run can still be considered valid.

3) Samples before and/or after a CCS that does not meet the QC Criteria should be considered suspect for the components in question. Any suspect samples should be reanalyzed by reinjection bracketed by a passing CCS.

H. Related Documents

1. NW63-0065 Collection and Preparation of Finished Goods Samples for Flavor Analysis by GCMS.
2. NW63-0066 Flavor Extraction and Analysis of Finished Goods Samples by GCMS.
3. FN64.0002RF GCMS Flavor Calibration Standards
4. ST-TM-410-634 Determination of Flavors in Smokeless Tobacco by GC-MS Validation Report, October 31, 2014.
5. ST-TM-410-634 Determination of Flavors in Smokeless Tobacco by GC-MS Transfer Report, August 7, 2015.

Title: Determination of Flavors in Smokeless Tobacco by GC-MS	Control #: ST-TM-410-634	Revision #: 1.0
	Approval Date: 09/22/2015	Effective Date: 10/1/2015
Test Method Owner: ALCS / RD&E / Analytical Technical Services		

I. Attachments

Attachment 1

Sample Chromatogram of a Calibration Standard

(b) (4)