Modification of Standardized Methods for the Measurement of Nicotine in Very Low Nicotine Content Cigarettes

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FDA – *Tobacco Product Standard for Nicotine Level of Combusted Cigarettes* – ANPRM

NICOTINE PRODUCT STANDARD ANPRM

FDA

- On March 15, FDA issued the *Tobacco Product Standard for Nicotine Level of Combusted Cigarettes*, an Advance Notice of Proposed Rulemaking (ANPRM)

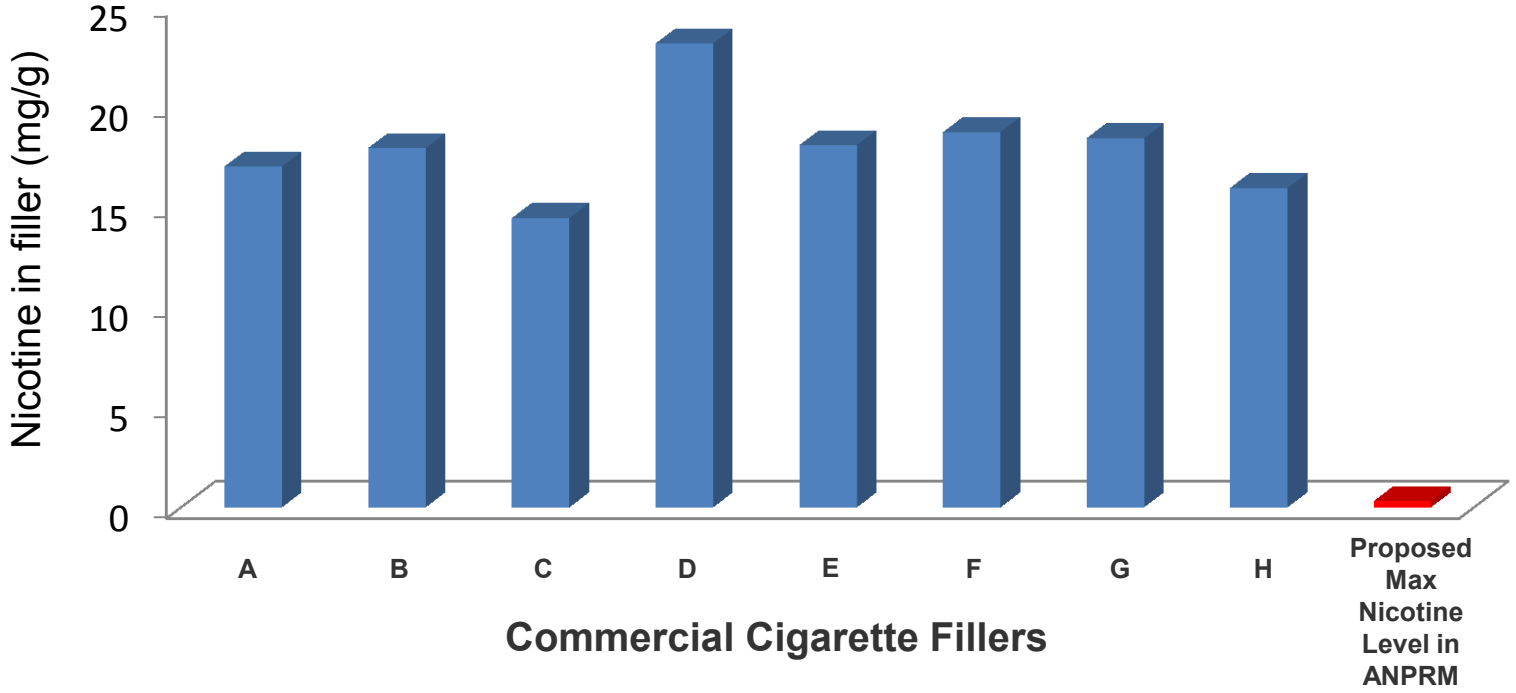
- Sought public comment for consideration in developing a potential product standard to lower nicotine to a minimally or non-addictive level in cigarettes
  - What potential maximum nicotine level would be appropriate for the protection of the public health;
  - How a maximum nicotine level should be measured;
  - Whether such a product standard should be implemented all at once or gradually;
  - Whether a nicotine product standard should also cover additional combustible tobacco products; and
  - What unintended consequences might occur as a result of such a standard

FDA – *Tobacco Product Standard for Nicotine Level of Combusted Cigarettes* – ANPRM

- Potential Scope:

- Potential Maximum Nicotine Level for cigarette tobacco filler: 0.3 - 0.5 mg/g
- Potential Analytical Testing Method

Commercial Cigarette Fillers vs Proposed Maximum Nicotine Level in ANPRM



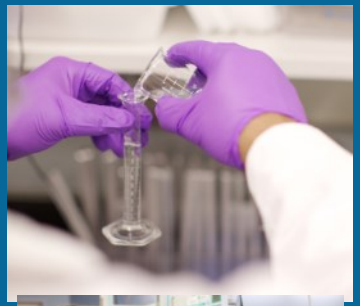
Analytical Testing Method – ANPRM

- FDA asked: Should the proposed tobacco product standard specify a method for the analysis of nicotine?
- FDA believes the test method should:
  1. Provide consistent results across accredited testing facilities
  2. Demonstrate a high level of specificity, accuracy, and precision to measure a range of nicotine levels across a wide variety of tobacco blends and products

Three Pillars of Effective Analytical Testing

Consensus Standardized Methods

Laboratory Proficiency Testing



Certified Tobacco Reference Materials

List of Analytical Testing Methods – ANPRM

12 methods referenced in ANPRM

- CRM 35, Continuous Flow Analysis (Filler)
- GC-FID (Filler)
- GC-MS confirmatory method (Filler)
- GC-MS using SPME (Filler)
- Other formats of GC-FID (Filler)
- Capillary electrophoresis using UV detection (Filler)
- SFC Chromatography-Ion Mobility Detection (Filler)
- Reverse phase ion-pair LC extraction (Filler)
- HPLC-UV (Filler)
- WHO TobLabNet, SOP 04, GC-FID (Filler)
- CRM 62, GC-FID (Filler)
- ISO 10315, GC-FID (Smoke)

Only two consensus standardized test methods referenced in ANPRM

- CRM 87, GC-MS (Filler) published 2018 – after ANPRM issued – is a consensus standardized method

Standardized Methods for Nicotine

Filler

CRM Nº 62: 2005

Determination of nicotine in tobacco and tobacco products by gas chromatographic analysis

CRM Nº 87: 2018

Determination of nicotine in tobacco products by GC-MS

Smoke

ISO 10315: 2013

Determination of nicotine in smoke condensates - Gas chromatographic method

Are Very Low Nicotine Content (VLNC) cigarette filler and smoke condensates within method scope?

Determination of Nicotine in Tobacco Filler

- Methods
  - CRM Nº 62 – GC-FID
    - Calibration range 5.0 – 40 mg/g
  - CRM Nº 87 – GC/MS
    - Calibration range 0.64 – 64 mg/g
- Repeatability and reproducibility values
  - Determined through interlaboratory collaborative studies
  - Range of nicotine levels in a variety of traditional tobacco products: 8 – 40 mg/g
- Maximum nicotine level proposed in ANPRM: 0.3 – 0.5 mg/g

Method scope and r and R values are not valid for VLNC filler

ALCS Modifications of CRM Nº 62 and CRM Nº 87

Parameter	CRM 62	Modified CRM 62	CRM 87	Modified CRM 87
LOQ (mg/g)	5.0	0.02	0.64	0.064
Calibration range (mg/g)	5 to 40	0.02 to 40	0.64 to 64	0.064 to 64
Injection mode	split	splitless	split	split
Regression model	equal	1/x	1/x	1/x
Sample mass : Extract volume	1g:50ml	1g:50ml	0.25g:40ml	0.25g:40ml

Modified method calibration ranges will bracket VLNC filler

Validation Results – ALCS Modifications

Parameter	Modified CRM 62 MTBE	Modified CRM 62 Hexane	Modified CRM 87
LOQ (mg/g)	0.02	0.02	0.064
S:N ratio	19	34	152
% Deviation at LOQ (n=6)	3%	9%	14%
Instrument precision at LOQ (n=6)	1.2%	0.9%	1.1%
Calibration range (mg/g)	0.02 to 40	0.02 to 40	0.064 to 64
Linear regression (r^2)	≥ 0.995	≥ 0.995	≥ 0.995

SRM NIST 3222 Cigarette Tobacco Filler

- Prepared from air cured, low nicotine tobacco
  - ❑ Processed and supplied by 22nd Century Group, Inc.
  - ❑ Developed through NIST and FDA Center for Tobacco Products
- Certified value for nicotine: 0.117 ± 0.018 mg/g
  - ❑ Based on 5 distinct data sets from NIST and CDC
    - ❑ 4 Methods LC/MS/MS
    - ❑ 1 Method GC/MS Method: 0.137 ± 0.016 mg/g
    - ❑ Range of test results: 0.095 – 0.137 mg/g with %RSDs 3%-17%
- Available through NIST

1. Sander L.C., Pritchett J.S, Daniles Y.C., Wood L.J., Lang B.E., Wise S.A., Development of a Cigarette Tobacco Filler Standard Reference Material, Anal. Chem., 2017 Vol. 89, 10461-10467.

Sample Results – ALCS Modifications

SRM NIST 3222[a] Air cured, low nicotine filler	CRM 62 MTBE	CRM 62 Hexane	CRM 87
Nicotine (mg/g) “as-is” (n=15)	0.154 ± 0.009	0.149 ± 0.019	0.170 ± 0.015
%Recovery from Fortification (n=3)	94%	90%	88%
Intermediate Precision (n=15)	5.8%	12.6%	8.9%
RT1[b] 1R6F cigarette filler	**CRM 62 MTBE**	**CRM 62 Hexane**	**CRM 87**
Nicotine (mg/g) “as-is” (n=9)	17.5 ± 0.2	17.7 ± 0.1	19.2 ± 0.3
%Recovery from Fortification (n=3)	98%	98%	95%
Intermediate Precision (n=9)	1%	< 1%	3%

[a] Standard reference material NIST 3222, VLNC cigarette tobacco filler 0.117 ± 0.018 mg/g
[b] RT1 reference material data sheet, 1R6F ground filler 18.905 ± 0.503 mg/g

Standardized Methods for Nicotine

Filler

CRM Nº 62: 2005

Determination of nicotine in tobacco and tobacco products by gas chromatographic analysis

CRM Nº 87: 2018

Determination of nicotine in tobacco products by GC-MS

Smoke

ISO 10315: 2013

Determination of nicotine in smoke condensates - Gas chromatographic method

Are Very Low Nicotine Content (VLNC) cigarette filler and smoke condensates within method scope?

SPECTRUM® Cigarettes

- 23 Different cigarette configurations
  - ❑ Manufactured by 22nd Century Group, Inc.
  - ❑ Distributed by Research Triangle Institute
- Reported range of nicotine in filler[1] 0.28 – 25 mg/g
- Reported range of nicotine in smoke[2] 0.01 -1.68 mg/cigt
- Available through NIDA's drug supply program
  - ❑ SPECTRUM® cigarettes are not available to the tobacco industry

1. Richter P., Pappas S., Bravo R., Lisko L.G., et al., Characterization of SPECTRUM Variable Nicotine Research Cigarettes, Tob. Regul. Sci.; 2016, 2(2), 94-105.
2. Ding Y.S., Richter P., Hearn B., et al., Chemical Characterization of Mainstream Smoke from SPECTRUM Variable Nicotine Research Cigarettes, Tob. Regul. Sci., 2017, 3 (1), 81-94.

Determination of Nicotine in Smoke

- Method
  - ISO 10315 – GC-FID
    - Calibration range 0.08 – 8.00 mg/cigt
- Repeatability and reproducibility values
  - Determined through interlaboratory collaborative studies
  - Range of nicotine in a variety of traditional cigarettes: 0.09 – 1.41 mg/cigt
- SPECTRUM® Cigarettes
  - Lowest nicotine yield[1]: 0.01 mg/cigt

Method scope and r and R values are not valid for VLNC smoke condensates

1. Ding Y.S., Richter P., Hearn B., et al., Chemical Characterization of Mainstream Smoke from SPECTRUM Variable Nicotine Research Cigarettes, Tob. Regul. Sci., 2017, 3 (1), 81-94.

ALCS Modifications of ISO 10315

Method	ISO 10315	Modified ISO 10315
LOQ (mg/cigt)	0.08	0.01
Calibration range (mg/cigt)	0.08 to 8	0.01 to 2
Injection mode	split	split
Regression model	equal	1/x
Y-intercept	include	ignore
Extraction volume	20 ml	10 ml

Modified method calibration range brackets VLNC cigarette smoke condensates

Validation Results – ALCS Modifications

Parameter	Modified ISO 10315
LOQ (mg/cigt)	0.01
S:N ratio at LOQ (n=30)	15.9
% Deviation at LOQ (n=6)	2%
Instrument precision at LOQ (n=6)	< 1%
Calibration range (mg/cigt)	0.01 to 2.00
Linear regression (r^2)	≥ 0.995

ISO Smoke Yields – ALCS Modifications

NIST 3222 cigarette filler was used to make handmade cigarettes

NIST 3222 (n=5) Air cured, low nicotine filler	TPM (mg/cigt)	Nicotine (mg/cigt)	%RSD
High Yield	10.5	0.019	8%
Med Yield	6.1	0.013	17%
Low Yield[a]	3.3	BLOQ	BLOQ
1R6F[b]	10.4	0.717	5%

[a] Below limit of quantitation; 0.01 mg/cigt.
[b] 1R6F Certified Reference Cigarette, Nicotine 0.721 ± 0.067 (mg/cigt)

Conclusions

- Existing standardized methods are not fit for the analysis of VLNC tobacco products
- Standardized methods can be modified for the analysis of nicotine in VLNC tobacco products
- Future steps
  - Create VLNC reference products
  - Standardize methods that include VLNC products
  - Establish proficiency testing for VLNC products

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