

ISO/TC 126/WG 24 – Nicotine Pouches

Updating Scope of ISO 21766:2021, *Tobacco and tobacco products – Determination of tobacco-specific nitrosamines in tobacco products – Method using LC-MS/MS* to include nicotine pouches

N-nitrosoanabasine (NAB)

N-nitrosoanatabine (NAT)

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

N-nitrosornicotine (NNN)

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Current ISO Standards for Analysis of TSNAs Include:

- 1) ISO 23921:2020 – MSS w/ an intense smoking regime
- 2) ISO 19290:2021 – MSS
- 3) ISO/TS 22304:2008 – Method using alkaline dichloromethane extraction
- 4) ISO 21766:2021 – Tobacco and tobacco products

The GothiaTek® standard was developed by Swedish Match to set limits on undesirable substances in their snus products. It reports that “some smokeless tobacco products, such as Swedish snus, are considered to be associated with substantially fewer health hazards than cigarettes”¹.

The GothiaTek® maximum level for TSNAs (total) in Swedish snus is 10 µg/g (DWB).

Data from several CORESTA collaborative studies, which cover a variety of tobacco products, are included in ISO 21766:2021. The range of total TSNAs in smokeless tobacco reference products were between 1.4 µg/g in snus (CRP1) to 18.3 µg/g in dry snuff (CRP3) ^{2,3}.

1. Rutqvist, L.E., Curvall, M., Hassler, T. *et al.* Swedish snus and the GothiaTek® standard. *Harm Reduct J* 8, 11 (2011).
2. CORESTA Smokeless Tobacco Sub-Group Technical Report, Working Group 2, 2009 Collaborative study report, July 2010, updated January 2016.
3. CORESTA Smokeless Tobacco Sub-Group Technical Report - CORESTA Reference Products 2015 Analysis, November 2015.

Current method limit of quantitation (LOQ) for ISO 21766:2021 is approximately 4 ng/g for NAB and 15 ng/g for NAT, NNK and NNN.

This is suitable for quantitation of TSNAs in tobacco matrices – ground tobacco, cigarette and cigar fillers and smokeless tobacco products (i.e., pouched snus, moist & dry snuff, chewing tobacco, etc.), where concentrations are typically >LOQ.

Nicotine pouches contain nicotine (synthetic or tobacco-derived), flavoring and other ingredients, but do not contain tobacco leaf, dust, or stem.

Concentrations of TSNAs in nicotine pouches are lower than the current calibration curve (if present at all).

The current LOQ yields total TSNA values of approximately 0.049 µg/g (well below the GothiaTek® maximum level). Reducing the LOQ of each TSNA for analysis of nicotine pouches will allow testing laboratories to perform more accurate assessments of total TSNA reduction when compared to traditional oral products, combustible cigarettes, etc.

Modifications can be made to the existing ISO standard to:

- 1) Include the nicotine pouch matrix
- 2) Reduce the LOQ for all matrices or establish an alternative calibration range specific for analysis of nicotine pouches.

Alternatively, a new standard can be developed specific to the analysis of trace level TSNA in nicotine pouches.

Swedish Match “Extended Calibration”

Swedish Match has extended the lower end of the calibration range and suggest using a lower concentration of internal standard and modified UPLC parameters to increase resolution between NAB and NAT-d4. The modified instrument parameters not only reduce instrument run time but reduce LOQs to 0.5 ng/g for each TSNA.

Table 3. Concentration and preparation of TSNA calibration standards

Cal. Std.	Volume of intermediate std.		Volume of IS	Volume of Acetonitrile	Conc. NNN	Conc. NNK	Conc. NAT	Conc. NAB
	no. 2	no. 3	2000 ng/mL	(mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
	(mL)	(mL)	(mL)					
Cal L1		0.085	1.00	22	0.0068	0.0068	0.0068	0.0017
Cal L2		0.200	1.00	22	0.016	0.016	0.016	0.0040
Cal L3		2.000	1.00	22	0.16	0.16	0.16	0.040
Cal 1	0.125		1.00	22	0.50	0.50	0.50	0.125
Cal 2	0.250		1.00	22	1.0	1.0	1.0	0.250
Cal 3	0.50		1.00	22	2.0	2.0	2.0	0.50
Cal 4	1.00		1.00	22	4.0	4.0	4.0	1.0
Cal 5	2.00		1.00	22	8.0	8.0	8.0	2.0
Cal 6	5.00		1.00	21	20	20	20	5.0
Cal 7	10.0		1.00	19	40	40	40	10
Cal 8	25.0		1.00	15	100	100	100	25

Reynolds American “Trace-Level” Analysis



RAIS reduced the LOQ to 0.375 ng/g for NAB and 1.5 ng/g for NAT, NNK, NNN) and suggest using a decreased internal standard concentration along with modified UPLC parameters to reduce instrument run time (6 minutes vs. 10 minutes).

Instrument Gradient.

Time (min)	% Mobile Phase A	% Mobile Phase B
0.0	100.0	0.0
1.7	100.0	0.0
3.5	70.0	30.0
4.5	40.0	60.0
5.0	100.0	0.0
6.0	100.0	0.0

Standard Concentration (ng/mL, NAB-NAT, NNK, NNN)	Solution used	Amount (μL)	Internal Standard (Spiking Solution) (μL)	Acetonitrile (mL)	100 mM Ammonium Acetate (mL)	Solution Label
0	N/A	0	0	10	10	TSNA-00
0	N/A	0	62	10	10	TSNA-0
0.0125-0.05 ng/mL	TSNA Stock Standard	25	124	20	20	TSNA-01
0.025-0.1 ng/mL	TSNA Stock Standard	25	62	10	10	TSNA-02
0.1-0.4 ng/mL	TSNA Stock Standard	100	62	10	10	TSNA-03
0.25-1.0 ng/mL	TSNA Stock Standard	250	62	10	10	TSNA-04
1.0-4.0 ng/mL	TSNA Stock Standard	1000	62	10	10	TSNA-05
2.5-10 ng/mL	TSNA Stock Standard	2500	62	10	10	TSNA-06
7.5-30 ng/mL	Purchased TSNA Custom Mix	75	62	10	10	TSNA-07
25-100 ng/mL	Purchased TSNA Custom Mix	250	62	10	10	TSNA-08
0.1-0.4 ng/mL	TSNA ICV Stock Standard	100	62	10	10	ICV

Market Assessment



Total TSNA LOQ (µg/pouch, as-is)						
	0.3 g pouch	0.4 g pouch	0.5 g pouch	0.6 g pouch	0.7 g pouch	0.8 g pouch
SM	0.00060	0.00080	0.00100	0.00120	0.00140	0.00160
RAIS	0.00146	0.00195	0.00244	0.00293	0.00341	0.00390

RAIS analyzed 146 commercially available nicotine pouch products

- 111 were <LOQ for all TSNA
- 35 were >LOQ for at least one TSNA
 - A consumer would have to use between 117 and 4762 pouches to reach GothiaTek® maximum acceptable levels*

*Assumes the pouches have a moisture content of 47.5%; total TSNA concentration on a dry-weight basis estimated to range between 0.0021 and 0.0857 µg/g

Discussion and Next Steps

ISO 27166:2021 is suitable for determination of TSNAs in tobacco and tobacco product matrices.

An extended calibration curve with a modified procedure for sample extraction/separation should be either:

- 1) Added to ISO 27166:2021
- 2) Added as a new ISO Standard, which details a new procedure for the extraction and quantitation of TSNAs in nicotine pouches.

Proposal is to execute a collaborative study using a combination of method parameters from Swedish Match and RAIS, assess the results reported from participating labs, and make a recommendation for either amending ISO 27166:2021 or creation of a new standard.

Thank You!

Questions and Open Discussion