

# Evaluation of Novel, Oral Tobacco-Derived Nicotine Products for HPHCs

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## Abstract

In May 2016, the U.S. Food and Drug Administration (FDA) issued a final rule to deem e-cigarettes, cigars and all other tobacco products to be subject to the Federal Food, Drug, and Cosmetic Act (the FD&C Act), as amended by the Family Smoking Prevention and Tobacco Control Act (Tobacco Control Act). Manufacturers of regulated tobacco products are required to report to FDA quantities of Harmful and Potentially Harmful Constituents (HPHCs) by November 8, 2019. FDA has not issued specific guidance for reporting HPHCs for novel oral tobacco products, such as those containing tobacco derived nicotine, as they have for certain other regulated tobacco products. Absent specific guidance from FDA, we measured HPHCs in VERVE<sup>®</sup> (oral, non-dissolvable, tobacco derived nicotine products) according to the requirements for smokeless tobacco, recognizing that these products do not meet the statutory definition of a smokeless tobacco product. The objective of this work was to modify and validate existing analytical methods to measure HPHCs in two product variants. An overview of the challenges and solutions that transpired during method validation for these unique matrices is provided. Also, the HPHC results are compared to other commercially available oral tobacco products and an oral nicotine replacement therapy (NRT) product. Results show the absence of detectable levels or significant reductions in HPHCs compared to traditional oral tobacco products and comparable HPHC results to the NRT.

## Background

- This study establishes the level of HPHCs in VERVE<sup>®</sup> Discs and VERVE<sup>®</sup> Chews and also provides a comparison to other tobacco products as required under section 910(b)(1)(A) of the FD&C Act.
- VERVE<sup>®</sup> Discs and Chews do not contain tobacco and their matrices present unique challenges to measure HPHCs commonly found in tobacco.
- The VERVE<sup>®</sup> Disc matrix is insoluble in many commonly used organic solvents. We were able to determine that milling the polymer to <1.25 mm allowed us to use solvents that are similar to those used for our tobacco matrices.
- The VERVE<sup>®</sup> Chews matrix is also insoluble in many commonly used organic solvents. We found it necessary to use an aqueous/organic combination and break down the matrix using a Geno/Grinder<sup>®</sup> to extract and subsequently measure some of the organic compounds in the abbreviated HPHC list.
- Studies were conducted to assess the HPHCs of VERVE<sup>®</sup> products over a twelve-month period in conditions consistent with ICH guidelines for products intended to be stored at ambient temperatures.
- The samples were stored in commercial packaging in environmental chambers set to 25°C ± 2°C / 60% relative humidity [RH] ± 5%.

## Methods

### Nicotine (total and free)

- VERVE<sup>®</sup> Discs are milled to <1.25 mm using a Wiley<sup>®</sup> Mill and an aliquot is extracted by vortexing for 1 hour in a methanol solution containing internal standard.
- VERVE<sup>®</sup> Chews are sonicated in water, then methanol is added along with internal standard before vortexing for 1 hour to extract the nicotine. After centrifuging, an aliquot of the extracts is prepared for analysis.

### pH

- VERVE<sup>®</sup> Discs are milled to <1.25 mm using a Wiley<sup>®</sup> Mill and an aliquot of the sample is diluted with deionized water and stirred. The pH of the aqueous extract is then measured at 5 minutes.
- VERVE<sup>®</sup> Chews are flattened in a pasta roller and diluted with deionized water and stirred. The pH of the aqueous mixture is measured at 5 minutes.

### Arsenic and Cadmium

- VERVE<sup>®</sup> Discs and Chews are digested without prior sample size reduction in nitric acid on a CEM<sup>®</sup> MARS 6<sup>®</sup> microwave digestion system and analyzed on a NexION<sup>®</sup> 350D ICP-MS.

### Benzo[a]pyrene

- VERVE<sup>®</sup> Discs are milled to <1.25 mm using a Wiley<sup>®</sup> Mill and an aliquot is extracted in acetonitrile and water on a Geno/Grinder<sup>®</sup> for 3 minutes at 1500 RPM. QuEChERS<sup>®</sup> salts are added and mixed thoroughly. A portion of the acetonitrile layer is extracted into hexanes and an aliquot is filtered and analyzed on an Agilent<sup>®</sup> 7890/5977 GC/MS equipped with a DB-17MS column.
- VERVE<sup>®</sup> Chews are extracted in acetonitrile and water on a Geno/Grinder<sup>®</sup> for 3 minutes at 1500 RPM. QuEChERS<sup>®</sup> salts are added and mixed thoroughly. A portion of the acetonitrile layer is extracted into hexanes and an aliquot is filtered and analyzed on an Agilent<sup>®</sup> 7890/5977 GC/MS equipped with a DB-17MS column.
- Due to interferences in the acetonitrile portion of the QuEChERS<sup>®</sup> salts extract, a back extraction was necessary to eliminate the interferences and achieve the desirable LOD and LOQ.

### NNN & NNK

- VERVE<sup>®</sup> Discs are milled to <1.25 mm using a Wiley<sup>®</sup> Mill and an aliquot is extracted in water/methanol containing internal standards using a Geno/Grinder<sup>®</sup> for 10 minutes at 1500 RPM. An aliquot of the extract is further purified and concentrated using solid phase extraction (SPE) to achieve the desired LOD and LOQ. Analysis is conducted on a Waters<sup>®</sup> ACQUITY UPLC<sup>®</sup> and Waters<sup>®</sup> Xevo<sup>®</sup> TQD using electrospray ionization.
- VERVE<sup>®</sup> Chews are cut into small pieces and extracted in water/methanol containing internal standards using a Geno/Grinder<sup>®</sup> for 10 minutes at 4000 RPM. An aliquot of the extract is further purified and concentrated using solid phase extraction (SPE) to achieve the desired LOD and LOQ. Analysis is conducted on a Waters<sup>®</sup> ACQUITY UPLC<sup>®</sup> and Waters<sup>®</sup> Xevo<sup>®</sup> TQD using electrospray ionization.

### Acetaldehyde, Crotonaldehyde, & Formaldehyde

- VERVE<sup>®</sup> Discs are milled to <1.25 mm using a Wiley<sup>®</sup> Mill and an aliquot is extracted and derivatized in acetonitrile with 2,4-dinitrophenylhydrazine (DNPH) on a Geno/Grinder<sup>®</sup> for 1 minute at 500 RPM.
- VERVE<sup>®</sup> Chews are extracted in acetonitrile and water on a Geno/Grinder<sup>®</sup> for 2 minutes at 1500 RPM then derivatized in acetonitrile with DNPH.
- The derivatized extract is analyzed for the respective hydrazones on a Waters<sup>®</sup> ACQUITY UPLC<sup>®</sup> and Micromass<sup>®</sup> Quattro Premier<sup>™</sup> MS.

## Results<sup>1</sup>

HPHC (unit/portion)	Chews		Analytical Limits		Discs		Analytical Limits	
	Blue Mint	Green Mint	LOQ	LOD	Blue Mint	Green Mint	LOQ	LOD
Acetaldehyde (µg) CAS# 75-07-7	0.488 (0.034)	0.472 (0.071)	0.100	0.056	BLOQ	BLOQ	0.025	0.014
Arsenic (ng) CAS# 7440-38-2	20.7 (1.8)	21.5 (1.5)	20.3	6.70	ND	ND	5.08	1.68
B[a]P (ng) CAS# 50-32-8	ND	ND	5.0	2.0	ND	ND	5.0	2.0
Cadmium (ng) CAS# 7440-43-9	ND	ND	14.1	4.66	7.26 (1.93)	7.56 (1.03)	3.53	1.17
Crotonaldehyde (mg) CAS# 4170-30-3	BLOQ	BLOQ	0.100	0.040	ND	ND	0.025	0.010
Formaldehyde (mg) CAS# 50-00-0	0.393 (0.028)	0.602 (0.054)	0.100	0.064	0.859 (0.211)	0.861 (0.155)	0.025	0.016
Nicotine total (mg) CAS# 54-11-5	1.471 (0.028)	1.540 (0.032)	0.48	0.12	1.383 (0.039)	1.447 (0.020)	0.50	0.12
Nicotine free (mg) CAS# 54-11-5	0.055 (0.009)	0.079 (0.011)	NA	NA	0.553 (0.043)	0.580 (0.024)	NA	NA
NNK (ng) CAS# 64091-91-4	BLOQ	BLOQ	1.0	0.2	0.38 (0.02)	0.34 (0.04)	0.25	0.05
NNN (ng) CAS# 16543-55-8	ND	ND	2.0	0.6	0.95 (0.07)	1.48 (0.23)	0.5	0.2

ND=not detected; BLOQ=below limit of quantitation; NA=Not Applicable

<sup>1</sup> Data represent the maximum values (mean (95% CI)) measured during 12 months storage time.

## Product Comparisons

HPHC	Units/ Portion	VERVE <sup>®</sup> Maximum Levels <sup>1</sup>	Smokeless Tobacco (General <sup>®</sup> Snus) <sup>2,3</sup>	Cigarettes <sup>4</sup>	Nicorette <sup>®</sup> Fresh Mint <sup>™</sup> Gum
Acetaldehyde	µg	0.488	20.7	1690	BLOQ
Arsenic	ng	21.5	74.63	11.3	47.3
B(a)P	ng	ND	ND	20.5	ND
Cadmium	ng	7.56	179.2	114	50.7
Crotonaldehyde	µg	<0.1	0.685	59.2	ND
Formaldehyde	µg	0.861	5.55	91.2	BLOQ
Nicotine (Total)	mg	1.54	10.9	2.59	2.02
Nicotine (Free)	mg	0.58	5.03	NR	2.00
NNK	ng	0.38	304	145	ND
NNN	ng	1.48	1080	284	ND

ND=not detected; NR=not reported; BLOQ=below limit of quantitation

<sup>1</sup> Data represent the maximum values (mean (95% CI)) for different products analyzed during 12 months storage time. B(a)P levels were below the detection limit of 2.00 ng/portion.

<sup>2</sup> Data obtained from Stepanov et al., (2008) (Average levels), based on average levels reported for General<sup>®</sup> snus. Note that the observations were converted from µg/portion to ng/portion.

<sup>3</sup> Data for HPHCs not reported by Stepanov et al. (2008), i.e., arsenic and cadmium, therefore we include values reported for General<sup>®</sup> snus Original Portion from Borgerding et al. (2012). Note that the observations were converted from ng/g of tobacco to ng/portion (since General<sup>®</sup> snus contains 1 g of tobacco) and from dry weight basis to as-is basis using the moisture content reported (50.9%).

<sup>4</sup> Data represent the mean (S.D.) for Marlboro 100's Box cigarettes smoked under Health Canada smoking conditions (Oldham et al., 2014)(Appendix Table 2)).

## Observations & Conclusions

- Chemical analyses of VERVE<sup>®</sup> products demonstrated low levels of HPHCs. Tobacco specific nitrosamine (TSNA) levels, NNN and NNK, were below levels of analytical quantitation in the chew product format and only slightly above levels of analytical quantitation in the discs product format.
- Modifications to our existing smokeless tobacco methods were necessary due to the unique nature of the VERVE<sup>®</sup> matrices. Method modifications such as the use of a Geno/Grinder<sup>®</sup> and QuEChERS<sup>®</sup> salts resulted in methodology to accurately measure FDA's abbreviated list of smokeless HPHCs while achieving acceptable LODs and LOQs.
- The VERVE<sup>®</sup> products contain nicotine (~1.5 mg) at levels similar to nicotine polacrilex (NRT) gum (2 mg). While Nicorette<sup>®</sup> Gum is not a tobacco product and VERVE<sup>®</sup> is not intended for cessation indications, a comparison of HPHCs is relevant since both contain similar levels of USP-grade tobacco-derived nicotine and similar oral routes of administration. As noted above, we observe higher levels of arsenic in VERVE<sup>®</sup> Chews than cigarette smoke, however, arsenic levels were lower than those measured in Nicorette<sup>®</sup> Gum and General<sup>®</sup> Snus which have similar routes of exposure.
- A toxicological risk assessment was conducted for all quantifiable HPHCs (except nicotine), with an estimated daily exposure assumption of 100% bioavailability of the VERVE<sup>®</sup> maximum level from 16 portions/day (16 portions are in 1 tube of VERVE<sup>®</sup> Discs). When compared to an established regulatory value (i.e., California OEHHA No Significant Risk Levels or ICH Q3D Permissible Daily Exposures for elemental impurities), estimated daily exposure to each of the HPHCs was below the established lifetime regulatory limit for each analyte which suggests negligible toxicological concern for lifetime exposure to VERVE<sup>®</sup> products.

## References

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