Amendment - Section VIII.B.1.iii Scientific Studies and Analyses Storage Stability

This amendment replaces Section VIII.B.1.iii of STNs MR 0000159 and MR0000160 by updating all information, figures, and tables to include 12-month storage stability and water activity data.

Reason for Amendment:

Room temperature storage stability and water activity studies were on-going at Enthalpy Analytical. At the time the application was filed, 12-month data was not available. Data from accelerated storage conditions up to six months of storage indicated that the product did not change. Twelve-month storage at room temperature is now available and is included with this amendment. The data indicate that the products are stable for at least 1 year.

Confidentiality: No claim of confidentiality is being made on the information contained in this amendment.

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VIII. Scientific Studies and Analyses

B. Product Analysis

1. Nicotine in Tobacco

iii. Storage Stability

Samples of VLN™ King and VLN™ Menthol King were sent to Enthalpy Analytical, LLC for storage stability testing. There were two storage conditions:

1) Stored at the condition of 25°C ± 2 °C, 60% RH ± 5% RH for standard stability evaluation.
2) Stored at the condition of 40°C ± 2 °C, 75% RH ± 5% RH for accelerated stability evaluation.

The samples were analyzed for TNCO under ISO conditions. Nicotine and Oven Volatiles was measured on the tobacco filler. Twenty (20) replicates were performed for each TNCO analysis; Seven (7) replicates for filler nicotine; and three (3) replicates for Oven Volatiles.

The test plan is shown in Table VIII.B.1.iii-1. Storage stability test plan. (the zero-time point was the same for both conditions).

Table VIII.B.1.iii-1. Storage stability test plan.

Storage Condition	**Initial**	**1 Month**	**3 Months**	**6 Months**	**9 Months**	**12 Months**
25°C/60%RH	X		X	X	X	X
40°C/75% RH	X	X	X	X		
Reference	(Enthalpy Analytical 2018 ProjCode 0318-100 and 0518-504)	(Enthalpy Analytical 2018 ProjCode 0318-101 and 0618-500)	(Enthalpy Analytical 2018 ProjCode 0318-103 and 0818-502R1)	(Enthalpy Analytical 2018 ProjCode 0318-106)	(Enthalpy Analytical 2019 ProjCode 0318-109)	(Enthalpy Analytical 2019 ProjCode 0318-112)

Figure VIII.B.1.iii-1 shows the smoke nicotine yield under when the regular and menthol cigarettes are stored at 25°C and 40% relative humidity (normal conditions) and 40°C and 75% relative humidity (accelerated conditions). Figure VIII.B.1.iii-2 shows the nicotine content when the regular and menthol cigarettes are stored at 25°C and 40% relative humidity (normal conditions) and 40° C and 75% relative humidity (accelerated conditions). Three months under the accelerated conditions is equivalent to one year at room temperature. The study data may be found in Table VIII.B.1.iii-2 and Table VIII.B.1.iii-3. There are no real differences in the amount of nicotine in the filler or in the nicotine in smoke in the regular or menthol VLN™ product after 1 year at room

temperature storage conditions indicating that the VLN™ product is stable for at least one year under normal storage conditions.

Figure VIII.B.1.iii-1. Smoke nicotine yield under normal and accelerated storage conditions (mean ± sd).

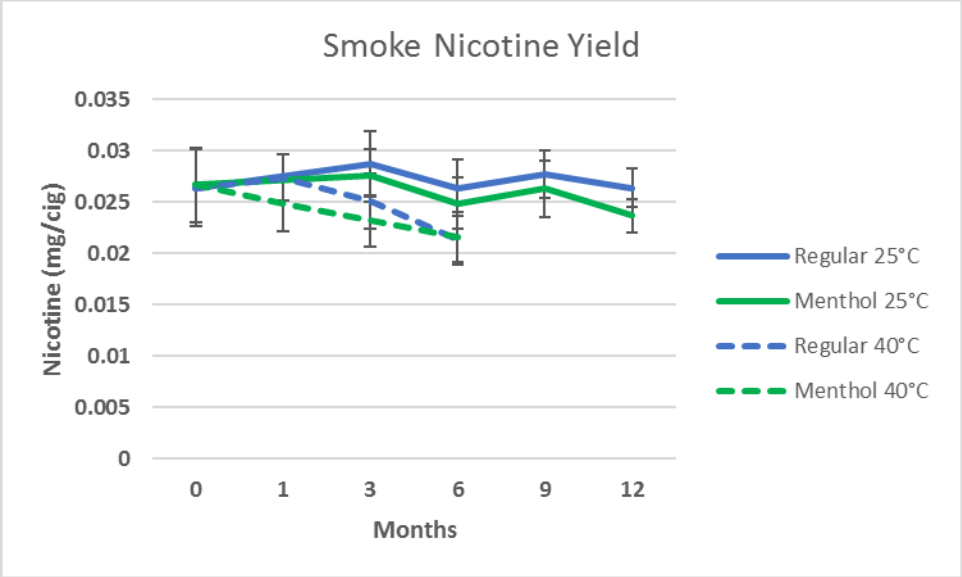


Figure VIII.B.1.iii-2. Tobacco nicotine content under normal and accelerated storage conditions (mean ± sd).

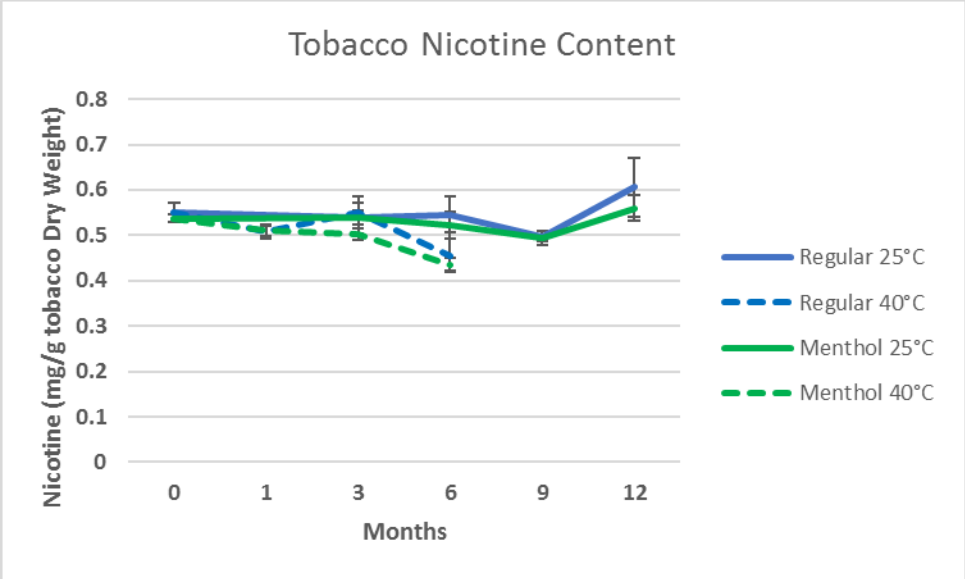


Table VIII.B.1.iii-2. Standard stability (25 °C ± 2 °C, 60% RH ± 5% RH).

Product	Analyte	Time Point (months)									
		0		3		6		9		12	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
VLN™ King	Tar (mg/cig)	7.04	0.36	7.14	0.45	7.11	0.39	6.64	0.44	6.81	0.34
	Nicotine(mg/cig)	0.0264	0.0037	0.0287	0.0032	0.0264	0.0027	0.0277	0.0023	0.0264	0.0019
	CO (mg/cig)	12.0	0.6	12.4	0.6	11.5	0.5	11.0	0.6	12.2	0.6
	Water (mg/cig)	0.449	0.167	0.638	0.106	0.354	0.136	0.362	0.135	0.482	0.106
	Puffs (#/cig)	5.81	0.14	5.87	0.10	5.56	0.17	5.54	0.22	5.81	0.21
	Filler Nicotine As Is (mg/g)	0.50	0.0190	0.483	0.0312	0.487	0.0350	0.442	0.00897	0.535	0.0572
	Filler Nicotine Dry Weight (mg/g)	0.551	0.0210	0.538	0.0349	0.546	0.0392	0.498	0.0101	0.606	0.0648
VLN™ Menthol King	Tar (mg/cig)	7.21	0.47	7.2	0.52	6.84	0.43	6.42	0.46	6.41	0.52
	Nicotine (mg/cig)	0.0267	0.0036	0.0276	0.0026	0.0249	0.0025	0.0263	0.0027	0.0237	0.0016
	CO (mg/cig)	12.0	0.5	12.1	0.7	10.7	0.8	10.36	0.63	11.1	0.71
	Water (mg/cig)	0.486	0.117	0.645	0.145	0.304	0.141	0.309	0.142	0.384	0.133
	Puffs (#/cig)	5.89	0.17	5.78	0.16	5.32	0.12	5.39	0.22	5.42	0.21
	Filler Nicotine As Is (mg/g)	0.471	0.00751	0.473	0.0134	0.460	0.0265	0.431	0.0133	0.491	0.0238
	Filler Nicotine Dry Weight (mg/g)	0.537	0.00856	0.538	0.0153	0.523	0.0301	0.493	0.0152	0.560	0.0271

Table VIII.B.1.iii-3. Accelerated Stability (40 °C ± 2 °C, 75% RH ± 5% RH)

Product	Analyte	Time Point (months)							
		0		1		3		6	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
VLN™ King	Tar (mg/cig)	7.04	0.36	7.72	0.60	6.45	0.37	6.44	0.58
	Nicotine(mg/cig)	0.0264	0.0037	0.0274	0.0022	0.0251	0.0027	0.0213	0.0024
	CO (mg/cig)	12.0	0.6	12.4	0.8	11.0	0.4	9.93	0.82
	Water (mg/cig)	0.449	0.167	0.438	0.171	0.56	0.159	0.362	0.217

Product	Analyte	Time Point (months)							
		0		1		3		6	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Puffs (#/cig)	5.81	0.14	5.90	0.18	5.52	0.16	5.18	0.20
	Filler Nicotine As Is (mg/g)	0.50	0.0190	0.450	0.0122	0.476	0.03	0.385	0.0308
	Filler Nicotine Dry Weight (mg/g)	0.551	0.0210	0.507	0.0138	0.550	0.0347	0.455	0.0364
VLN™ Menthol King	Tar (mg/cig)	7.21	0.47	7.49	0.48	6.51	0.5	6.62	0.41
	Nicotine (mg/cig)	0.0267	0.0036	0.0248	0.0026	0.0232	0.0025	0.0216	0.0024
	CO (mg/cig)	12.0	0.5	11.6	0.9	10.6	0.59	10.9	0.5
	Water (mg/cig)	0.486	0.117	0.394	0.160	0.593	0.129	0.365	0.166
	Puffs (#/cig)	5.89	0.17	5.77	0.29	5.44	0.19	5.25	0.18
	Filler Nicotine As Is (mg/g)	0.471	0.00751	0.443	0.0104	0.427	0.0118	0.366	0.0111
	Filler Nicotine Dry Weight (mg/g)	0.537	0.00856	0.511	0.0120	0.502	0.0118	0.436	0.0133

As part of the storage stability study, samples were analyzed for water activity by Enthalpy Analytical to determine if there was sufficient water for microbial growth. The results of the 12-month analysis are attached (Project Code 318-112).

Table VIII.B.1.iii-4 shows the samples analyzed. Table VIII.B.1.iii-5 list the results after storage at 25° C for up to 12 months. There was very little change. Under accelerated storage conditions of 40° C for six months (equivalent to 2 years at ambient conditions) there was a slight increase in water activity. The results are shown in Figure VIII.B.1.iii-3. Water activity Results under Ambient and Accelerated Storage Conditions. There was no difference in water activity between the menthol and regular product.

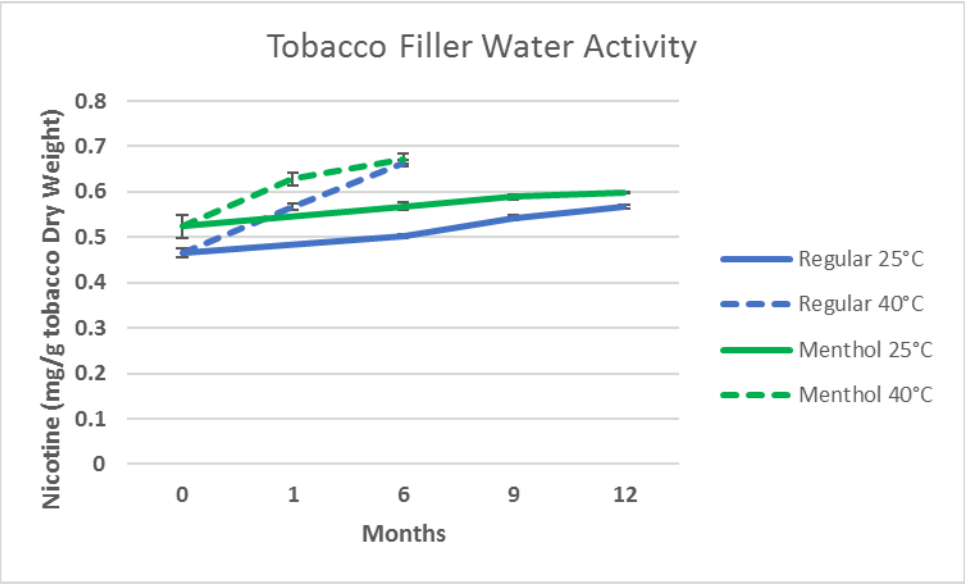
Table VIII.B.1.iii-4. Test Plan for Water Activity Samples

Storage Condition	**Initial**	**1 Month**	**6 Months**	**9 Months**	**12 Months**
25°C/60%RH	X		X	X	X
40°C/75% RH	X	X	X		
Reference	Enthalpy Analytical 2019 Project Code 0318-900	Enthalpy Analytical 2019 Project Code 0318-900	Enthalpy Analytical 2019 Project Code 0318-900	Enthalpy Analytical 2019 Project Code 0318-900	(Enthalpy Analytical 2019 Project Code 0318-112)

Table VIII.B.1.iii-5. Water Activity Results (mean ± standard deviation).

	Time Point (months)				
Sample/Storage Condition	**0**	**1**	**6**	**9**	**12**
VLN™ King 25°C	0.465 ± 0.010	-	0.502 ± 0.004	0.543 ± 0.006	0.567 ± 0.003
VLN™ Menthol King 25°C	0.524 ± 0.026	-	0.568 ± 0.009	0.589 ± 0.005	0.598 ± 0.001
VLN™ King 40°C	0.465 ± 0.010	0.568 ± 0.007	0.664 ± 0.007	-	-
VLN™ Menthol King 40°C	0.524 ± 0.026	0.628 ± 0.014	0.672 ± 0.011	-	-

Figure VIII.B.1.iii-3. Water activity Results under Ambient and Accelerated Storage Conditions.



Growing tobacco has minimal levels of TSNA's (Spiegelhalder and Fischer, 1991). It is generally recognized that TSNA's are formed during the curing process either by microbial enabled nitrosation or by nitrosation by direct heat curing. In the case of Vector 21-41 tobacco, the tobacco is air cured so TSNA formation could possibly occur after potential microbial growth. Water activity or a_w is the partial vapor pressure of water in a substance divided by the standard state partial vapor pressure of water. In the field of food science, the standard state is most often defined as the partial vapor pressure of pure water at the same temperature. Using this particular definition, pure distilled water has a water activity of exactly one. Higher a_w substances tend to support more microorganisms. Bacteria usually require at least 0.91, and fungi at least 0.7 (Rockland and Beuchat, 1987). The results of this study on VLN™ King and VLN™ Menthol King cigarettes made with Vector 21-41 tobacco show that the available water, even after 12 months, is insufficient for microbial growth and therefore TSNA's should not be increased. Mutasa et al. (1990) evaluated the relationship of water content and fungal spoilage in cured tobacco. Visible moulding occurred within 7-14 days at 0.85 – 0.90 a_w, but only after 6 months at 0.70 – 0.75 a_w (Mutasa et al, 1990). The water activity of

VLN™ cigarettes is well below the levels that support microbial growth, even after storage for 12 months. Accelerated storage studies suggest that this hold true for up to 2 years of storage.

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