

Preclinical Testing Of Flavors In E-vapor Products, Part 2: Preparation And Stability Characterization Of Representative Flavor Mixtures

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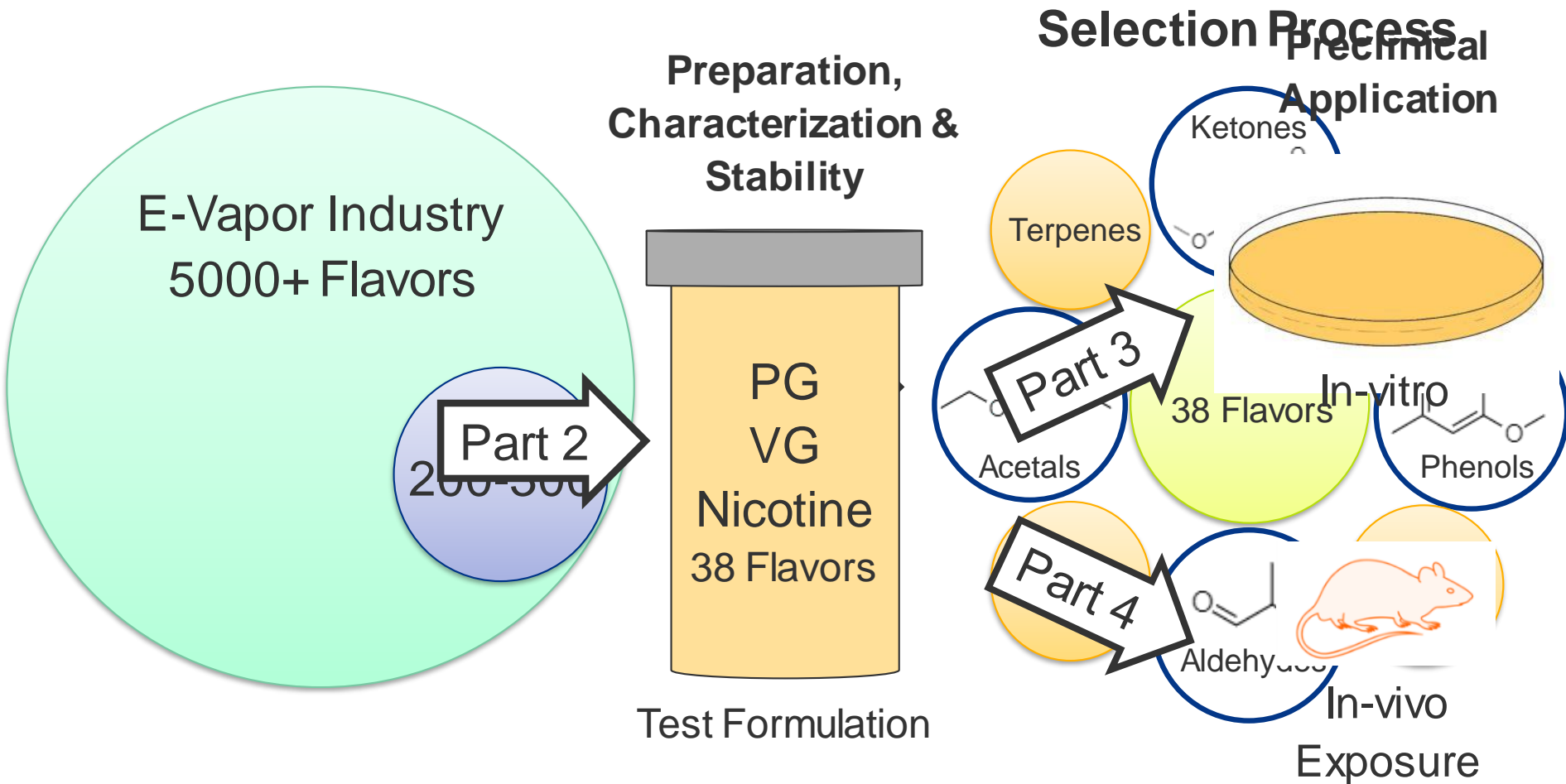
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Overview of Session

- Part 1: Selection of Representative Flavor Mixtures Using a Structural Grouping Approach (Kim Ehman)
- **Part 2: Preparation and Stability Characterization of Representative Flavor Mixtures (Cameron Smith)**
- Part 3: In Vitro Cytotoxicity and Genotoxicity of Representative Flavor Mixtures (Utkarsh Doshi)
- Part 4: Flavor Transfer from the Liquid to the Aerosol for Inhalation Exposure (Jingjie Zhang)

Preclinical Testing of Flavors in E-vapor Products: Overview



Objectives

- Maximize the shelf life of flavor mixtures to avoid repetitive and time consuming batch characterization necessary in preclinical studies
- Evaluate the effectiveness of our shelf life stability strategy using gas chromatography-mass spectrometry (GC-MS)

Definition: Pre-blends

- Basic concept: concentrated ingredients (flavors) are diluted and combined to make a final mixture or product
- Pre-blends (or concentrates) are commonly used in the food and beverage manufacturing industry due to prolonged shelf life, reduced packaging volume, and lower distribution and storage costs.¹
- **Pre-blends** used in this study are concentrated (5-20x more than the test formulation) mixtures containing PG, ethanol, and selected flavor compounds.

¹ She, M., & Hwang, S. T. (2006). *Journal of membrane science*, 279(1-2), 86-93.

Study Design

Longer Stability

Pre-blend I – 22

Pre-blend II – 7

Pre-blend III – 2

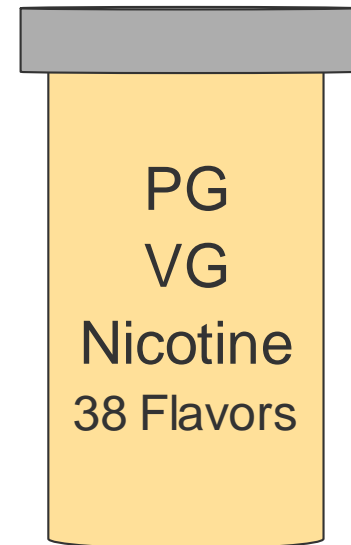
Pre-blend IV – 6

Pre-blend V – 1

Dilute with PG, VG,
Nicotine



Shorter Stability



**Test
Formulation**



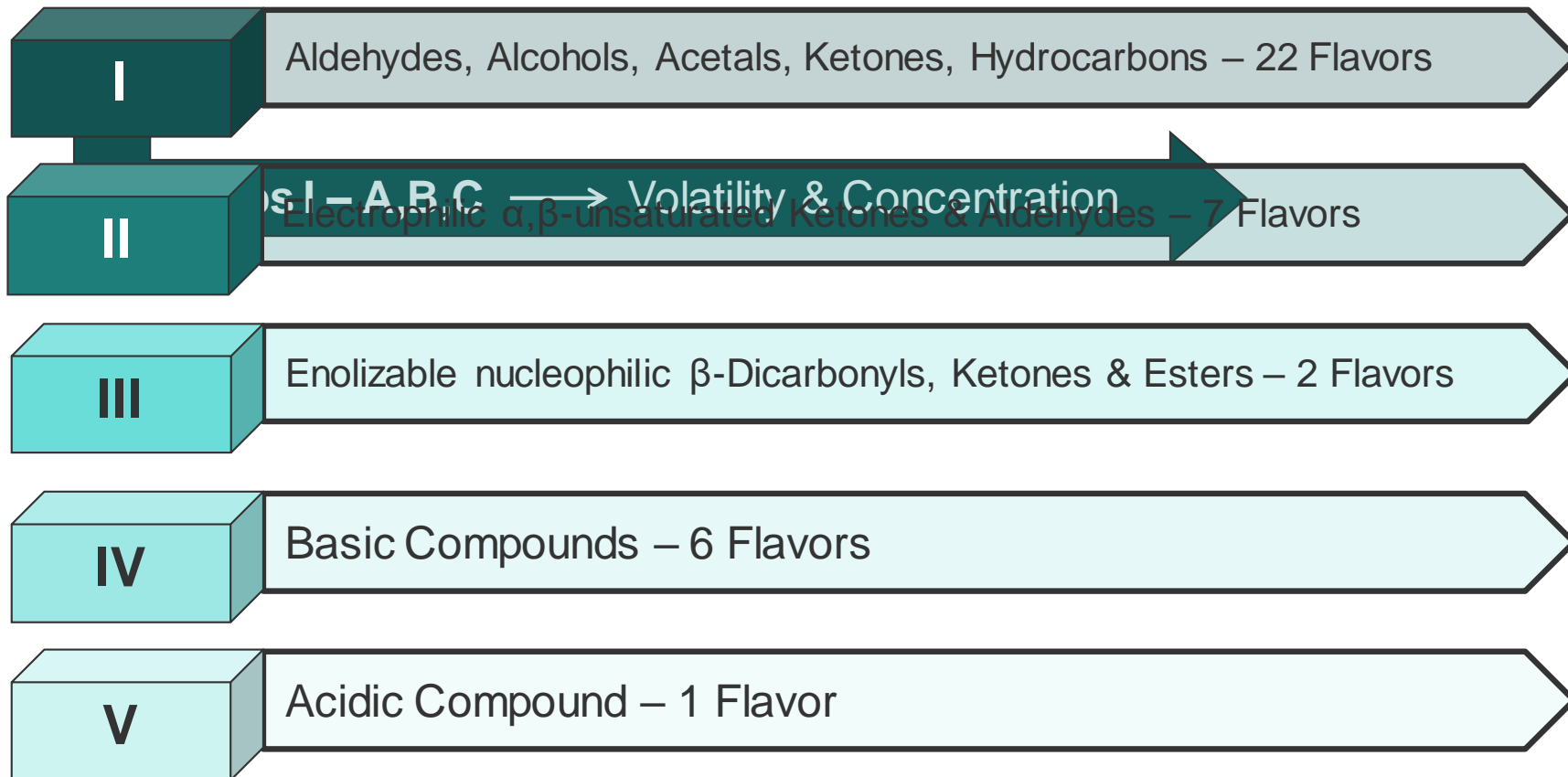
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Pre-blend Categorization

- Evaluated reactivity of compounds based on functional group characteristics
 - Alcohols < Aldehydes, Ketones & Esters < Enolizable Aldehydes & Ketones < α,β -unsaturated Ketones & Aldehydes < Acids and Bases
- Grouped compounds in minimum number of categories as possible to shorten and simplify preparation time of pre-blends
- Ensured compounds within each grouping had limited reactivity

Pre-blend Categories

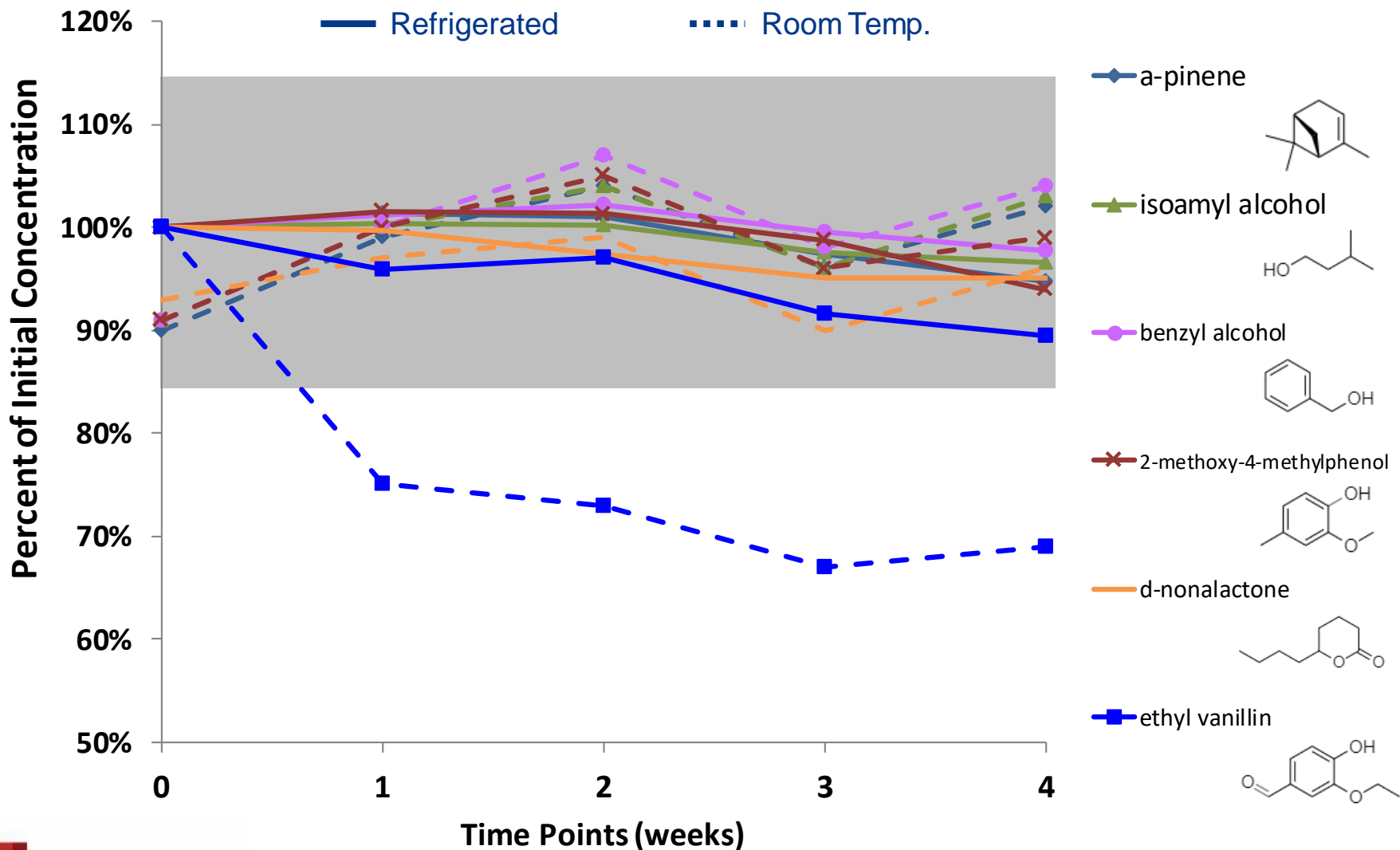


Study Design

- 1 Month Stability for Pre-blends
 - Refrigerated and Room Temperature Conditions
- 10 Days Stability for Test Formulations (All 38 Flavors)
 - Refrigerated and Room Temperature Conditions
- Acceptance Criteria
 - Pre-blends – $\pm 15\%$ of initial value
 - Test Formulations – $\pm 20\%$ of the initial value

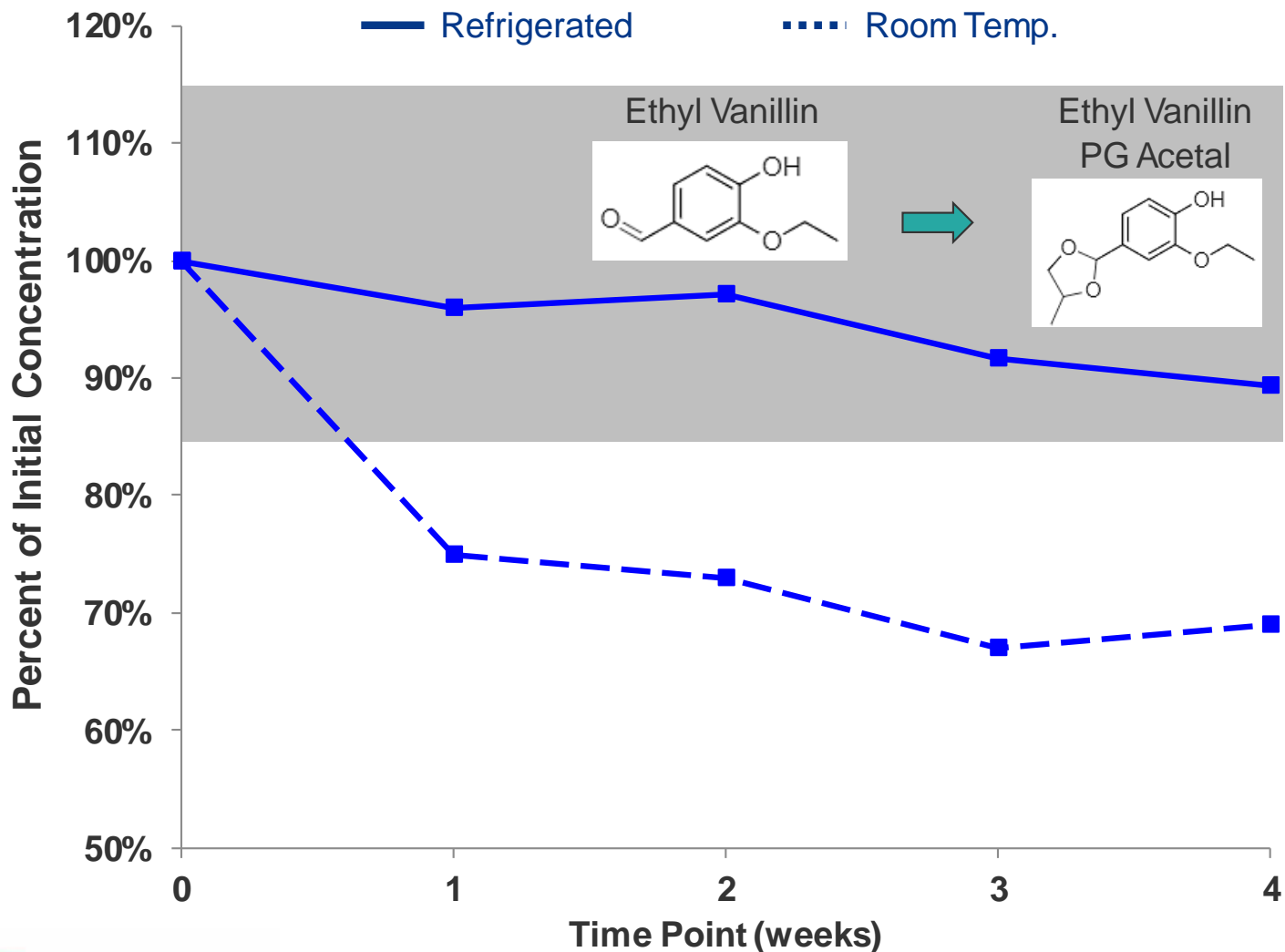
Pre-Blend IC

Aldehydes, Alcohols, Acetals, Ketones, Hydrocarbons



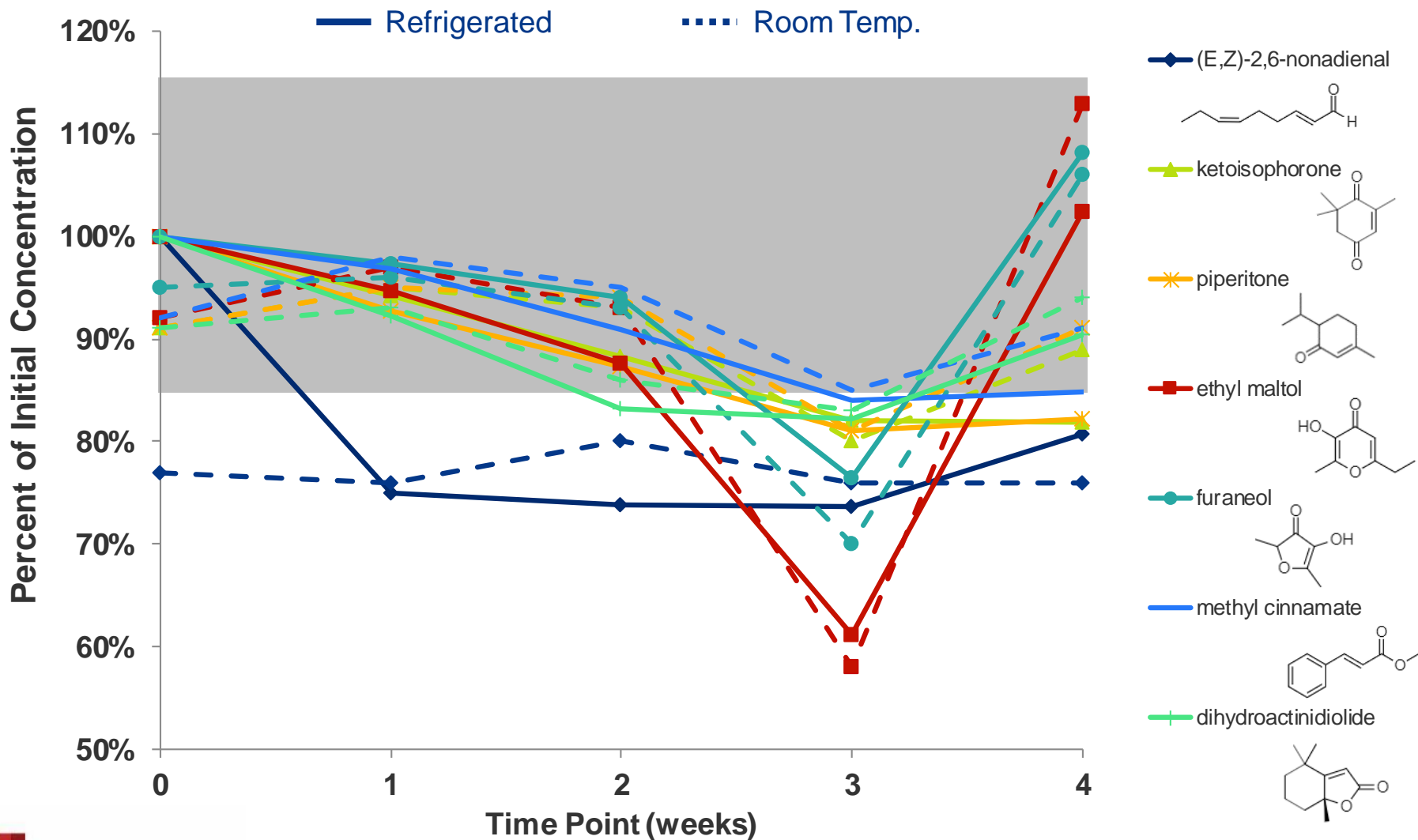
Pre-Blend IC

Ethyl Vanillin Stability



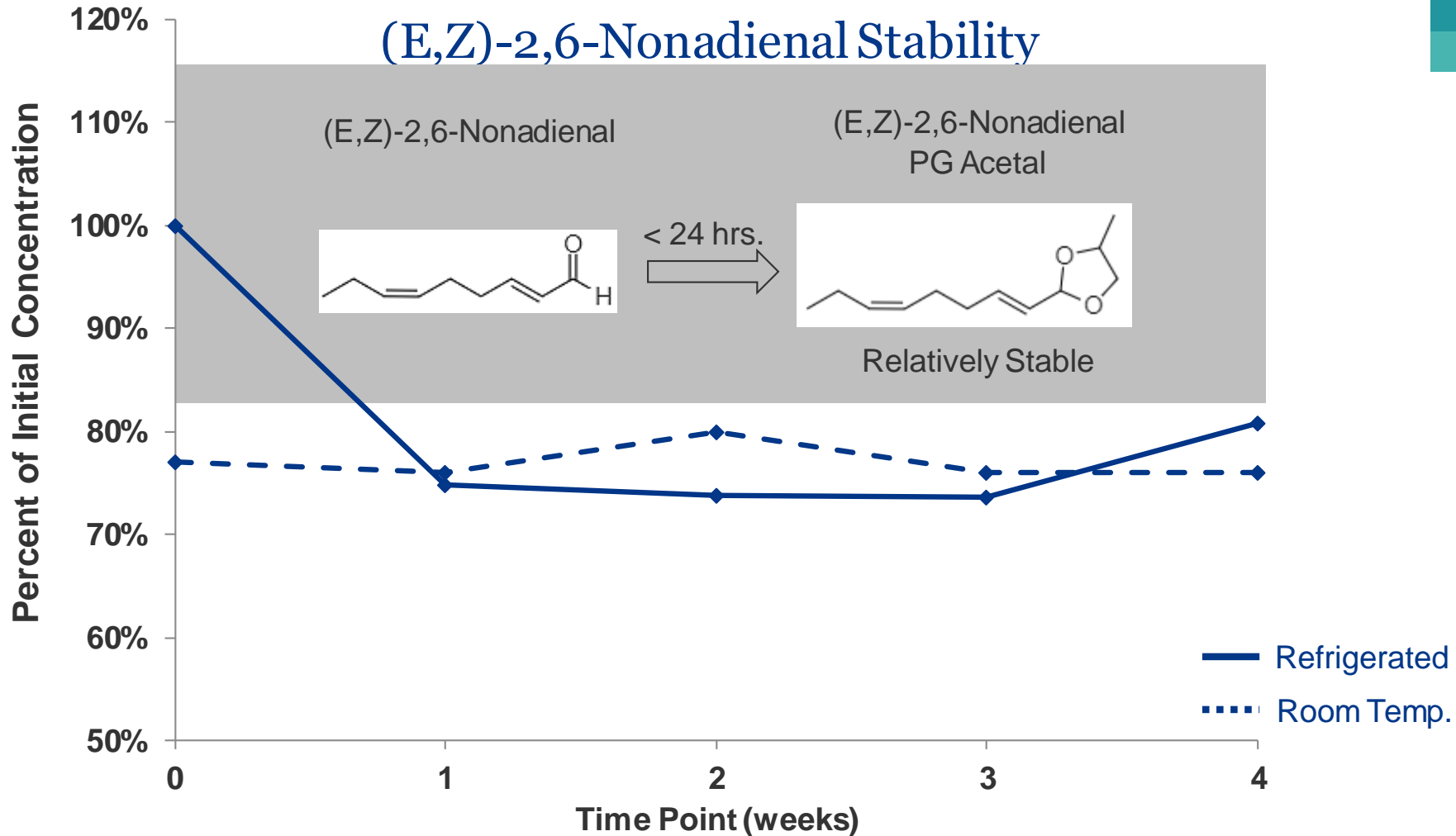
Pre-blend II

Electrophilic α,β -unsaturated Ketones & Aldehydes



Pre-blend II

(E,Z)-2,6-Nonadienal Stability

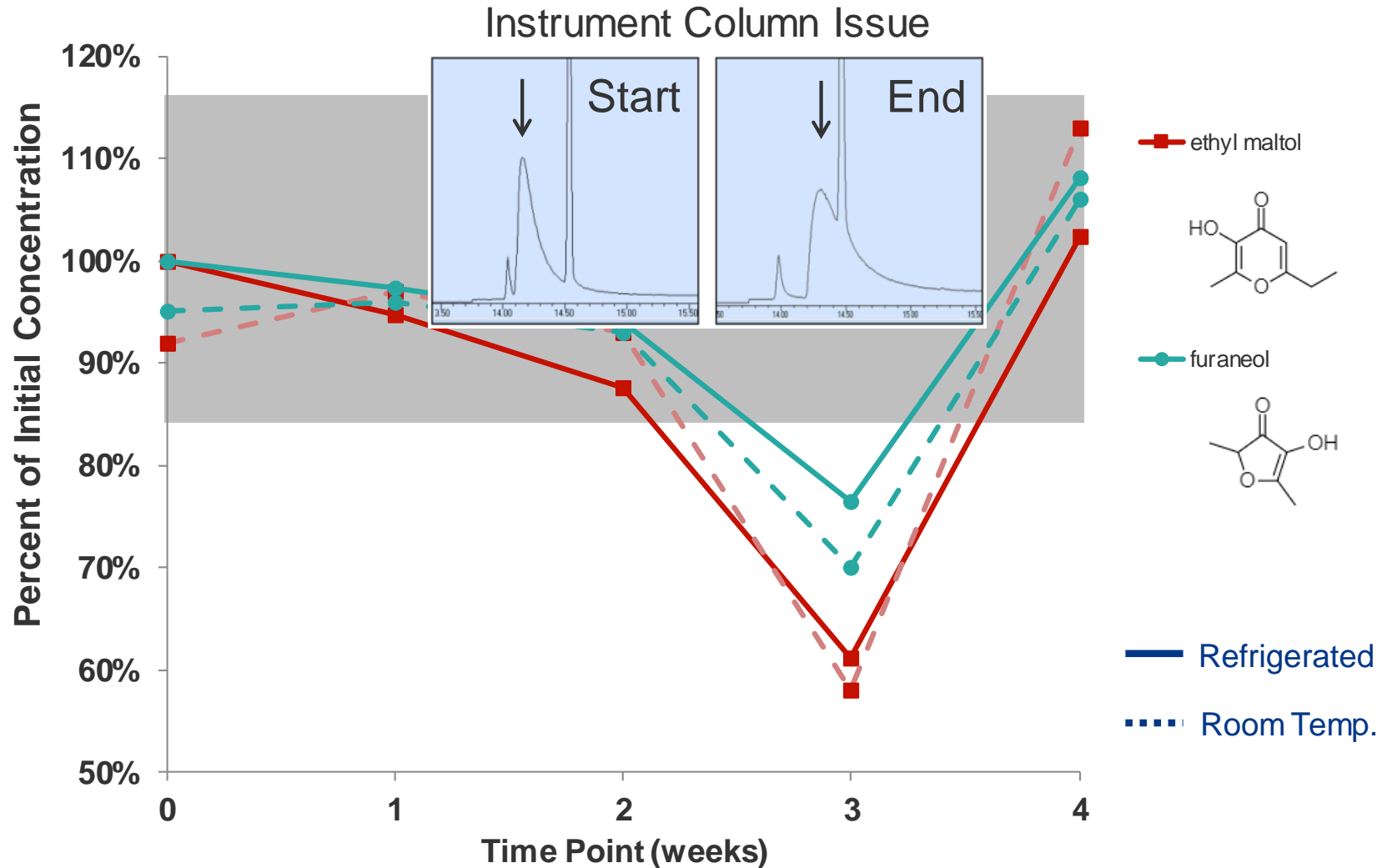


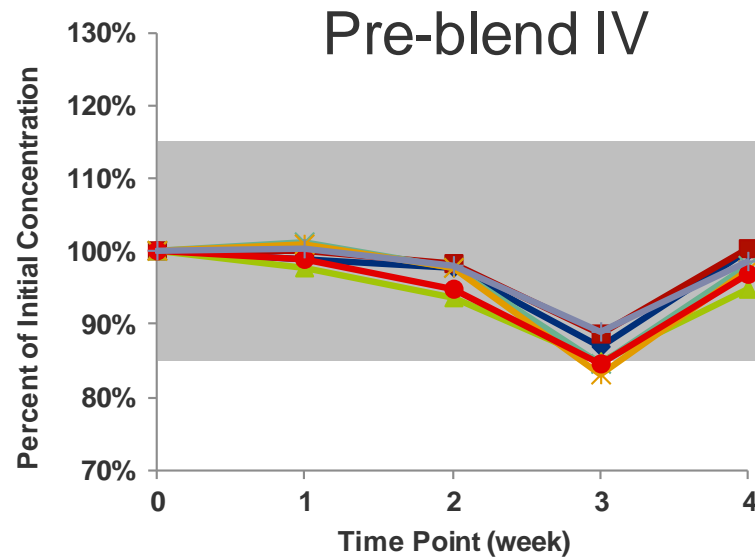
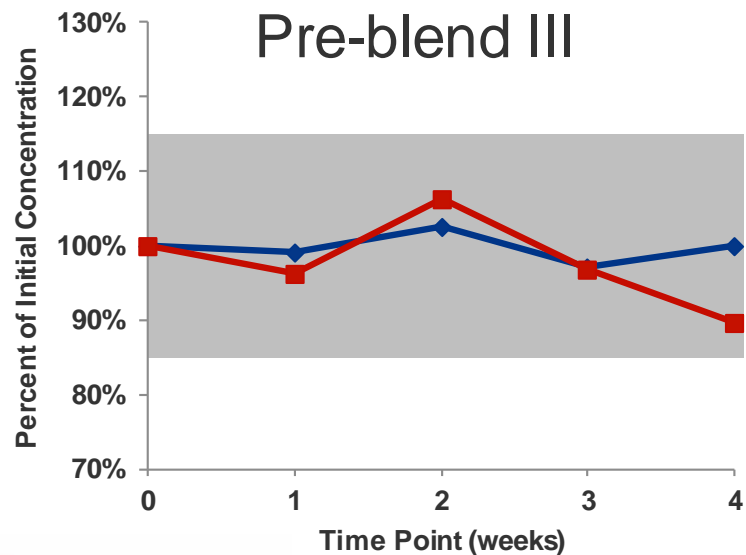
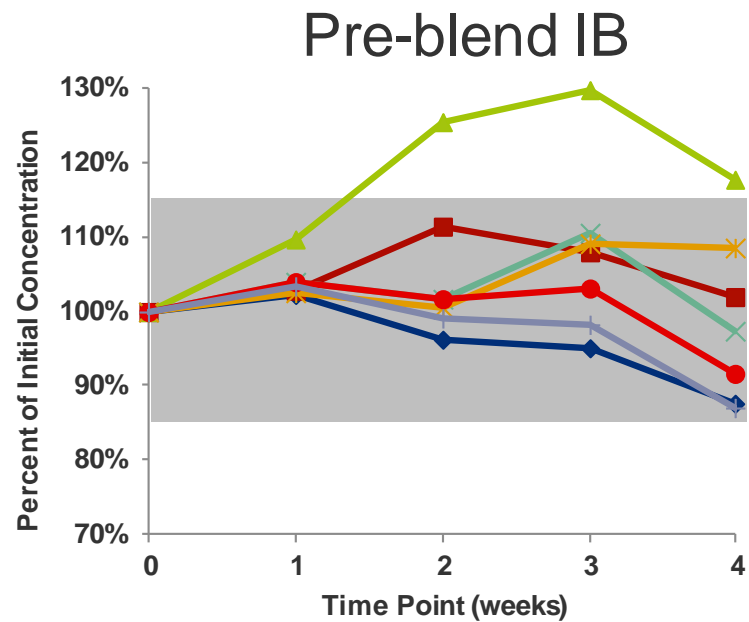
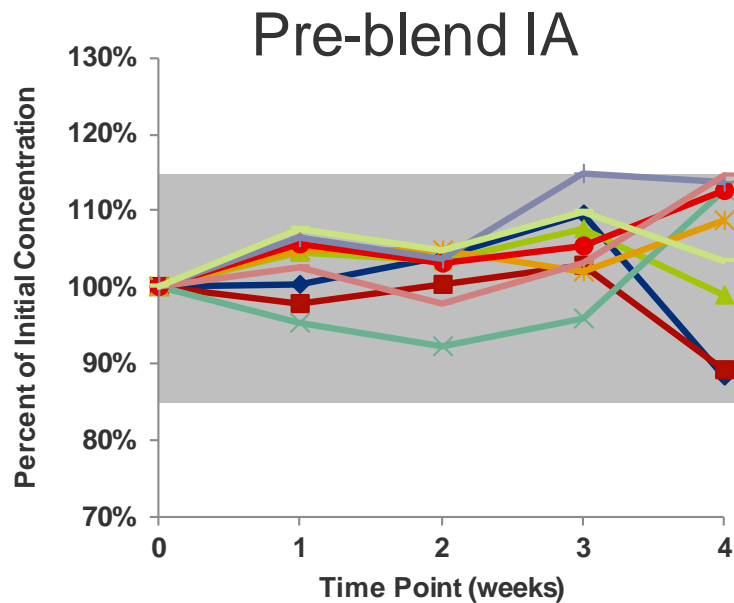
(E,Z)-2,6-Nonadienal Initial Time Point – **73% lower than expected** theoretical concentration
 All Other Flavors – Accuracy within $\pm 15\%$ of theoretical conc.



Pre-blend II

Ethyl Maltol & Furaneol Stability





Study Design

Longer Stability

Pre-blend I – 22

Pre-blend II – 7

Pre-blend III – 2

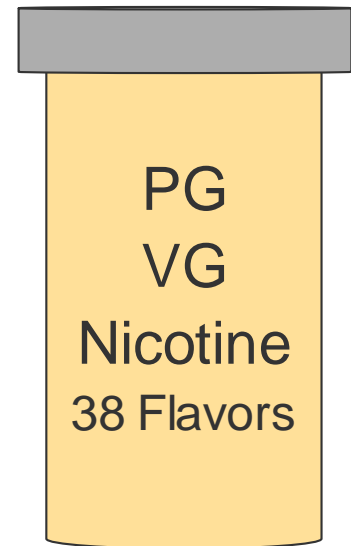
Pre-blend IV – 6

Pre-blend V – 1

Dilute with PG, VG,
Nicotine



Shorter Stability



**Test
Formulation**



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Test Formulation with Nicotine - Refrigerated

	T0	T1 - 1 day	T2 - 7 days (± 1 day)	T3 - 11 days (± 1 day)
Pre-blend IA Compounds				
p-cymene	100%	97%	96%	97%
1-penten-3-one	100%	93%	56%	45%
isopulegol	100%	95%	93%	94%
isobutyraldehyde	100%	88%	84%	91%
citronellol, D-L-	100%	96%	90%	91%
ethyl lactate	100%	96%	90%	94%
cis-3-hexenol	100%	97%	96%	93%
acetal	100%	111%	106%	107%
2-methyl-4-phenyl-2-butanol	100%	97%	98%	97%
Pre-blend IB Compounds				
ambrox (Cetalox®)	100%	98%	95%	94%
a-damascone (trans)	100%	96%	90%	89%
linalool	100%	90%	83%	81%
p-dimethoxybenzene	100%	96%	96%	94%
ethyl 2-methylbutyrate	100%	107%	106%	114%
acetanisole	100%	94%	92%	89%
eugenyl acetate	100%	98%	97%	95%
Pre-blend IC Compounds				
a-pinene	100%	103%	109%	105%
isoamyl alcohol	100%	101%	104%	104%
benzyl alcohol	100%	101%	104%	105%
2-methoxy-4-methylphenol	100%	101%	107%	106%
d-nonalactone	100%	99%	99%	99%
ethyl vanillin	100%	101%	106%	107%

	T0	T1 - 1 day	T2 - 7 days (± 1 day)	T3 - 11 days (± 1 day)
Pre-blend II Compounds				
(E,Z)-2,6-nonadienal	100%	94%	89%	79%
ketoisophorone	100%	100%	104%	104%
piperitone	100%	100%	106%	106%
ethyl maltol	100%	100%	111%	106%
furaneol	100%	96%	93%	86%
methyl cinnamate	100%	101%	107%	106%
dihydroactinidiolide	100%	101%	106%	106%
Pre-blend III Compounds				
3-methyl-2,4-nonanedione	100%	102%	105%	104%
triethyl citrate	100%	103%	109%	110%
Pre-blend IV Compounds				
2,5-dimethylpyrazine	100%	101%	106%	105%
3-ethylpyridine	100%	101%	106%	105%
2-acetylthiazole	100%	101%	108%	105%
p-mentha-8-thiol-3-one	100%	88%	73%	70%
2-acetylpyrrole	100%	102%	106%	106%
methyl anthranilate	100%	98%	96%	92%
Additional Compounds				
2-methylbutyric acid	100%	99%	107%	100%
nicotine	100%	97%	115%	102%

Test Formulation without Nicotine - Refrigerated

	T0	T1 - 1 day	T2 - 7 days (± 1 day)	T3 - 11 days (± 1 day)
Pre-blend IA Compounds				
p-cymene	100%	102%	104%	94%
1-penten-3-one	100%	99%	92%	81%
isopulegol	100%	103%	104%	88%
isobutyraldehyde	100%	106%	102%	86%
citronellol, D-L-	100%	100%	91%	82%
ethyl lactate	100%	95%	98%	92%
cis-3-hexenol	100%	99%	101%	87%
acetal	100%	102%	107%	95%
2-methyl-4-phenyl-2-butanol	100%	99%	99%	88%
Pre-blend IB Compounds				
ambrox (Cetalox®)	100%	99%	96%	95%
a-damascone (trans)	100%	101%	96%	95%
linalool	100%	93%	90%	86%
p-dimethoxybenzene	100%	96%	93%	92%
ethyl 2-methylbutyrate	100%	100%	104%	105%
acetanisole	100%	95%	90%	89%
eugenyl acetate	100%	97%	95%	95%
Pre-blend IC Compounds				
a-pinene	100%	101%	103%	100%
isoamyl alcohol	100%	98%	99%	98%
benzyl alcohol	100%	97%	101%	97%
2-methoxy-4-methylphenol	100%	98%	103%	98%
d-nonolactone	100%	96%	102%	96%
ethyl vanillin	100%	98%	105%	100%

	T0	T1 - 1 day	T2 - 7 days (± 1 day)	T3 - 11 days (± 1 day)
Pre-blend II Compounds				
(E,Z)-2,6-nonadienal	100%	98%	99%	92%
ketoisophorone	100%	97%	101%	97%
piperitone	100%	97%	102%	97%
ethyl maltol	100%	102%	110%	104%
furaneol	100%	97%	101%	96%
methyl cinnamate	100%	97%	103%	98%
dihydroactinidiolide	100%	96%	105%	97%
Pre-blend III Compounds				
3-methyl-2,4-nonanedione	100%	100%	105%	101%
triethyl citrate	100%	102%	114%	106%
Pre-blend IV Compounds				
2,5-dimethylpyrazine	100%	97%	97%	97%
3-ethylpyridine	100%	98%	98%	98%
2-acetylthiazole	100%	98%	97%	97%
p-mentha-8-thiol-3-one	100%	99%	92%	92%
2-acetylpyrrole	100%	98%	98%	98%
methyl anthranilate	100%	97%	92%	92%
Additional Compounds				
2-methylbutyric acid	100%	98%	97%	97%

Conclusion

- The use of pre-blends can substantially simplify the timely preparation and characterization necessary for preclinical testing
- Depending on the test formulation ingredients, pre-blends can be stable for a matter of months in refrigerated conditions
- All test formulation flavor ingredients used in the study were stable for at least 3 days in the presence of nicotine and 10 days without nicotine
- Test article characterization and stability assessment are recommended

Acknowledgements

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