

Introduction

- New e-cigarette refill solutions and pod systems have entered the market with claims to be free of tobacco-derived nicotine
- WARNING: This product contains tobacco-free nicotine. Nicotine is an addictive chemical.**
- WARNING: This product contains synthetic nicotine. Nicotine is an addictive chemical.**
- WARNING: This product contains nicotine not derived from tobacco. Nicotine is an addictive chemical.**
- Nicotine from tobacco is predominately in the S-isomeric form
- Synthetic nicotine typically contains racemic (50:50) mixture of the S- and R-isomer forms
- Along with nicotine, minor tobacco alkaloids (MTAs) are extracted from the tobacco plant, including:
 - Myosmine · Nornicotine · Anabasine · Anatabine
- Assessing tobacco-free nicotine claims are challenging given NMR is the only definitive technique currently available
- Our study aimed to compare **nicotine isomer ratios** and the **presence of MTAs** to suggest likely nicotine derivation in commercial e-cigarette products

Products

32 commercial refill solutions, 7 pod-systems, 1 tobacco-free nicotine base (100% PG) and 1 tobacco-derived nicotine base (50:50 PG:VG) were purchased from local vendors

Refill solutions	Pod-style products	Tobacco Free Nicotine Base (100mg/mL)	Tobacco-Derived Nicotine Base (100mg/mL)
31 with tobacco-free claims	5 with tobacco-free claims		
1 without tobacco-free claims	2 without tobacco-free claims		

Methods

50µL of each liquid added to 9.95mL of methanol

Nicotine Isomer Ratio Assay

20µL → 980µL of Extraction Solution (5 µg/mL Nicotine-d4 in Methanol)

1µL injected into Nextera X2 UHPLC (Shimadzu), 6500+ MS (Sciex)

Minor Tobacco Alkaloids (MTA) Assay

Reconstitute → 1mL of methanol

1µL injected into 7890B/7250 GC/Q-TOF (Agilent)

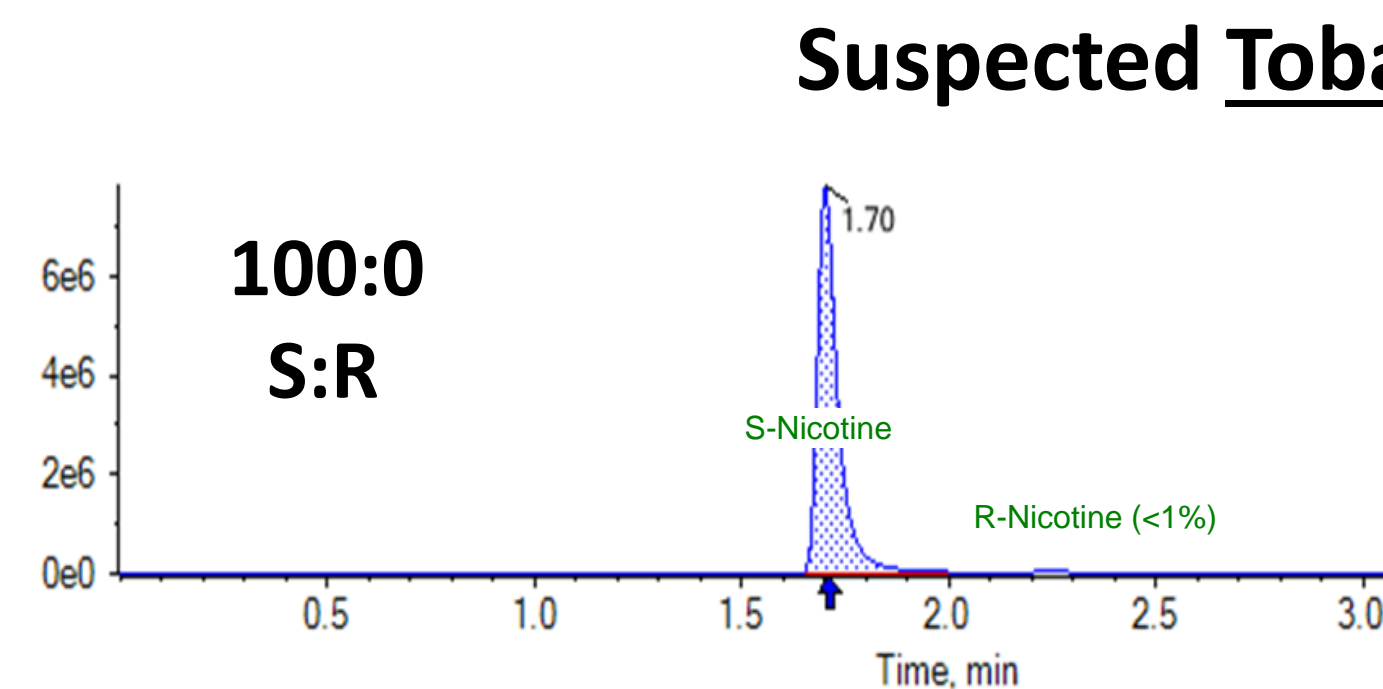
Method Parameters:

Column: DB-624UI 30m x 0.25mm x 0.25µm

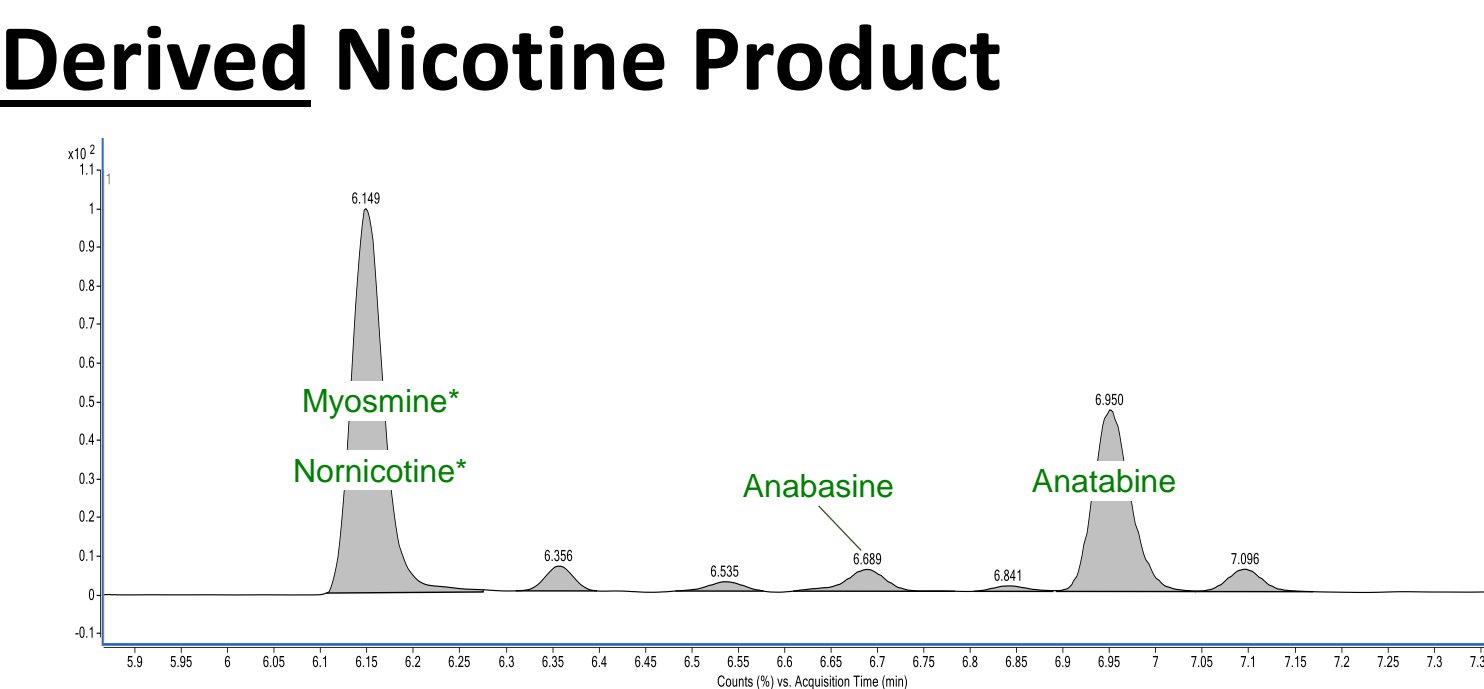
MRM Ions: Myosmine: 145.0703 → 117.0534
Nornicotine: 147.0868 → 105.0418
Anabasine: 161.1016 → 104.0462
Anatabine: 159.0861 → 130.0607

Chromatograms

Nicotine Isomer Ratio (NIR)



Minor Tobacco Alkaloids (MTA)



Suspected Synthetic-Derived Nicotine Product

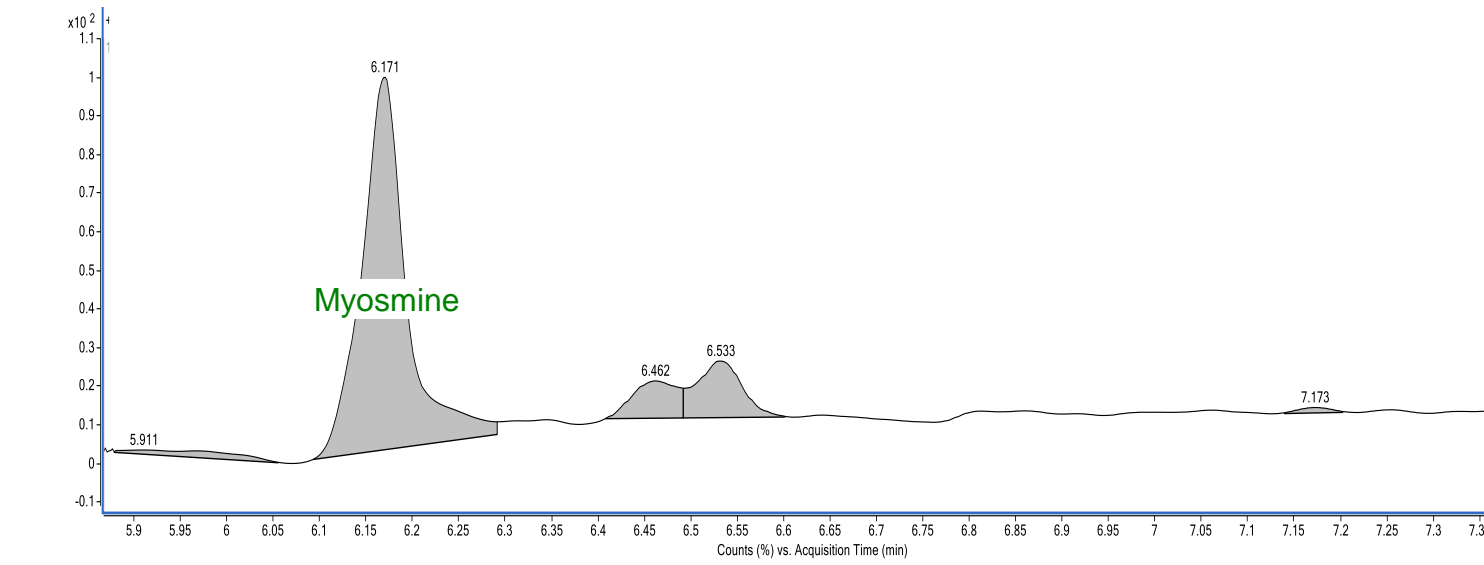
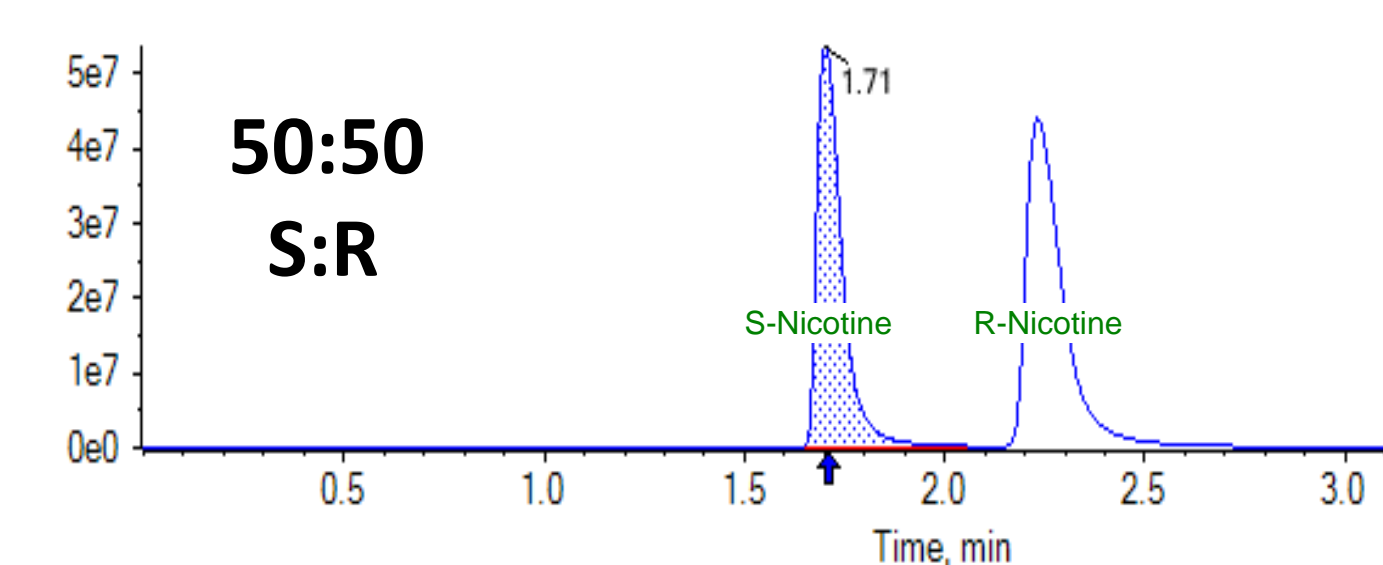
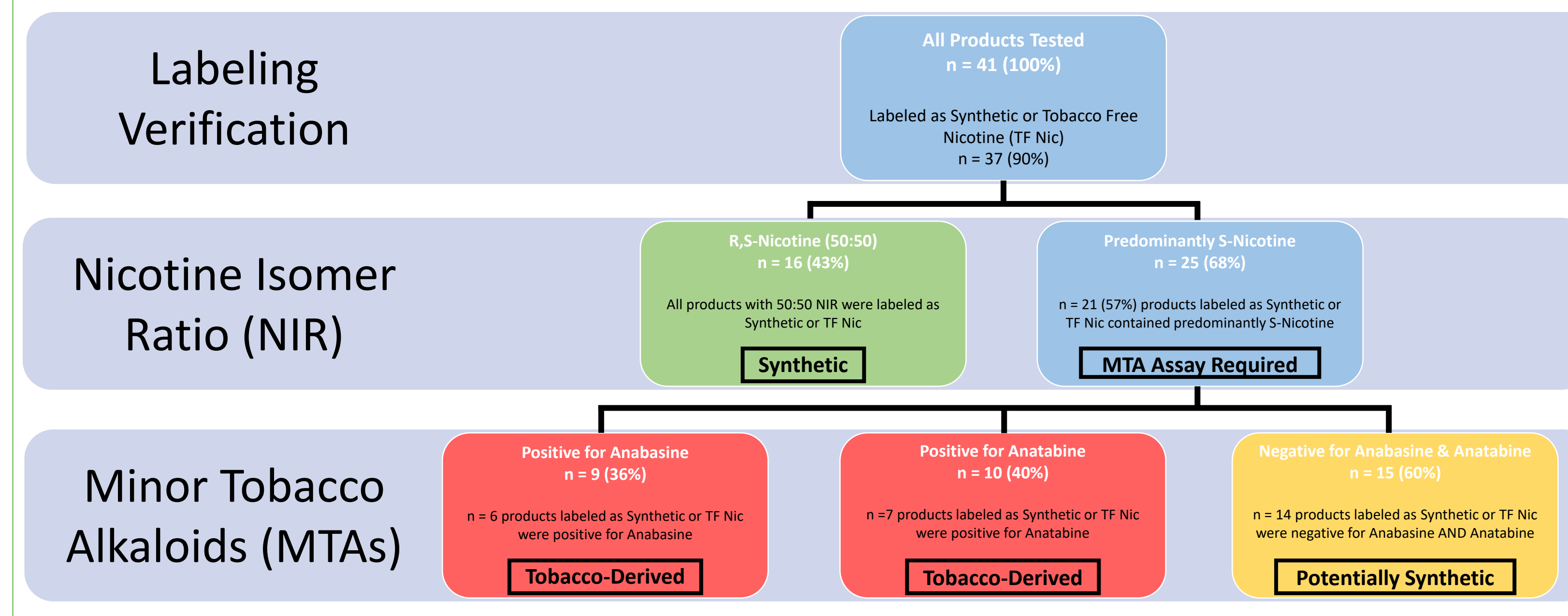


Figure 1 – S,R-nicotine ratio chromatograms for 2 commercial products

Figure 2 – MTA chromatograms for 2 commercial products. *Myosmine & nornicotine co-elute and are separated by unique MRM ions.

Figure 3 – Decision Tree to Determine Nicotine Source Using NIR and MTA Assays



Results

TF Nic Claim	Type	Brand	S:R Ratio (%)	MTA Detected				Accurate Claim
				Nornicotine	Myosmine	Anabasine	Anatabine	
No	Base	NicSelect (Tobacco-Derived Nicotine Base)	100:0	Yes	Yes	No	No	Maybe
	POD	Juul	100:0	Yes	Yes	Yes	Yes	✓
	E-liquid	Mighty Vapors Salt	100:0	Yes	Yes	Yes	Yes	✓
Yes	Base	Tobacco Free Nicotine (TFN) Base	50:50	Yes	Yes	No	No	✓
	POD	Pacha Mama	50:50	Yes	Yes	No	No	✓
		Ignite	50:50	Yes	Yes	No	No	✓
		Dinner Lady	50:50	Yes	Yes	No	No	✓
		Hyde N-bar	100:0	Yes	Yes	Yes	Yes	✗
	Hyde N-bar	90:10	No	Yes	Yes	Yes	✗	
	E-liquid	Naked 100 Salt	50:50	Yes	Yes	No	No	✓
		Glas Bx Salt	50:50	Yes	Yes	No	No	✓
		Coastal Clouds Salt	50:50	Yes	Yes	No	No	✓
		Glas	50:50	No	Yes	No	No	✓
		Orchard Blends by Five Pawns	50:50	No	Yes	No	No	✓
		Coastal Clouds	50:50	No	Yes	No	No	✓
		Directors Cut by Bad Drip	50:50	Yes	Yes	No	No	✓
		Cloud Nurdz Salt	50:50	No	Yes	No	No	✓
		FreeNoms	50:50	No	Yes	No	No	✓
Cloud Nurdz		50:50	No	Yes	No	No	✓	
Fryd		50:50	No	Yes	No	No	✓	
Juice Head	50:50	No	Yes	No	No	✓		
Aqua Drops Salts	100:0	Yes	Yes	No	No	Maybe		
Custard MONSTER Salt	100:0	No	Yes	No	No	Maybe		
Mr. Freeze SALT	100:0	No	Yes	No	No	Maybe		
Dinner Lady Salt	100:0	No	Yes	No	No	Maybe		
Pod Juice Salt	100:0	No	Yes	No	No	Maybe		
Phrut Salt	100:0	No	Yes	No	No	Maybe		
Keep It 100	100:0	No	Yes	No	No	Maybe		
Aqua Drops	100:0	No	Yes	No	No	Maybe		
Mr. Freeze	100:0	Yes	Yes	No	No	Maybe		
Killa Fruits	100:0	No	Yes	No	No	Maybe		
Custard MONSTER	100:0	No	Yes	No	No	Maybe		
Phrut	100:0	No	Yes	No	No	Maybe		
Air Factory	100:0	No	Yes	No	No	Maybe		
Dinner Lady	100:0	Yes	Yes	No	No	✗		
Kilo Revival Salt	100:0	No	Yes	Yes	Yes	✗		
Killa Fruits Salt	100:0	No	Yes	Yes	Yes	✗		
Air Factory Salt	100:0	Yes	Yes	No	Yes	✗		
Humble E-Liquid	100:0	No	Yes	Yes	Yes	✗		
Moo E-Liquids	100:0	Yes	Yes	Yes	Yes	✗		

Conclusions

- 43% of commercial products with synthetic nicotine claims had 50:50 S:R nicotine ratios, confirming synthetic derivation.
- Myosmine was found in all products, reducing the selectivity of this marker.
- Tobacco-derived products, but not the tobacco-derived or TFN nicotine bases, contained anabasine or anatabine.
 - Both markers were found only in products with 100:0 S:R nicotine ratios.
- This study suggests that presence of anabasine and/or anatabine in combination with 100:0 S:R isomers are possible indicators for tobacco-derived nicotine.
- In combination with both methods, 7 (19%) commercial products with synthetic claims likely contain nicotine-derived from tobacco.
- Additional markers or alternative methods are needed for 100:0 S:R products without anabasine or anatabine markers.

Disclosures

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