Quantitative and Qualitative Analysis of Dissolvable Tobacco Products

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Project Highlights

- Center for Tobacco Products (CTP) requested analysis
- Dissolvable tobacco products analyzed: Stonewall and Ariva tablets, Camel Orbs, Camel Sticks, Camel Strips, Marlboro Tobacco Sticks, and Skoal Tobacco Sticks
- Quantitative analysis for nicotine, pH, calculated free-base nicotine, and five tobacco-specific N-nitrosamines (TSNA), metals
- Qualitative survey of products for additional chemical constituents of interest


Smokefree Satisfaction
Ariva
20 Dissolvable Tobacco Pieces
Wintergreen
A Smokeless Tobacco Product
JAVA
STONEWALL
20 Dissolvable Tobacco Pieces
Spit-Free
Underage Sale Prohibited.
A Smokeless Tobacco Product
2001/203 (Star Scientific)
orbs
fresh
STICKS
Mellow
strips
Fresh
DISSOLVABLE TOBACCO
2009 (R.J. Reynolds Tobacco)
COOL MINT
TOBACCO
STICK
Marlboro
TEN SMOKELESS TOBACCO STICKS
disease and tooth loss
ORIGINAL
TOBACCO
STICK
SKOAL
WARNING:
Smokeless tobacco is
addictive
2011 (Altria/Phillip Morris)

Quantitative Results

Nicotine and pH analysis

Sample ID	Type	Wt (g)	pH	Nicotine (mg/g)	Nicotine (mg/piece)
Camel Strips Fresh	Strip	0.211	7.95	2.17	0.46
Camel Orbs Fresh	Tablet	0.258	7.88	3.9	1.01
Camel Orbs Mellow	Tablet	0.257	7.8	4.09	1.05
Skoal Rich	Tobacco Sticks	0.216	7.23	5.77	1.24
Marlboro Cool Mint	Tobacco Sticks	0.205	7.64	6.1	1.25
Marlboro Original	Tobacco Sticks	0.213	7.56	6.08	1.29
Marl Smooth Mint	Tobacco Sticks	0.219	7.71	5.97	1.31
Skoal Original	Tobacco Sticks	0.223	7.54	5.93	1.32
Skoal Smooth Mint	Tobacco Sticks	0.233	7.72	5.65	1.32
Skoal Mint	Tobacco Sticks	0.223	7.69	6.01	1.34
Marlboro Rich	Tobacco Sticks	0.216	7.49	6.2	1.34
Ariva Java	Tablet	0.267	7.46	5.7	1.52
Ariva WG	Tablet	0.267	7.57	5.81	1.55
Camel Sticks Mellow	Sticks	0.511	7.69	3.1	1.58
Stonewall WG	Tablet	0.463	7.23	8.34	3.86
Stonewall Natural	Tablet	0.466	7.39	8.6	4.00
Stonewall Java	Tablet	0.46	7.34	8.74	4.02
	AVERAGE	0.29	7.58	5.77	1.73
	MINIMUM	0.21	7.23	2.17	0.46
	MAXIMUM	0.51	7.95	8.74	4.02

TSNA Analysis

Sample ID	Type	NAB	NAT	NNK	NNN
			(ng/piece)		
Skoal Original	Tobacco Sticks	14.9	296	113	419
Skoal Rich	Tobacco Sticks	14.2	274	109	393
Skoal Mint	Tobacco Sticks	15.2	294	118	431
Skoal Smooth mint	Tobacco Sticks	14.8	301	119	425
Marlboro Original	Tobacco Sticks	14.1	272	104	375
Marlboro Rich	Tobacco Sticks	14.3	278	102	409
Marlboro Cool Mint	Tobacco Sticks	14.0	272	104	397
Marl Smooth Mint	Tobacco Sticks	14.9	278	106	406
Camel Strips Fresh	Strip	4.8	32.0	41.9	43.4
Camel Sticks Mellow	Sticks	12.5	84.5	133	113
Camel Orbs Mellow	Orbs	17.1	158	45.9	235
Camel Orbs Fresh	Orbs	28.9	292	52.7	381
Stonewall Wintergreen	Tablet	4.6	101	22.7	43.5
Stonewall Java	Tablet	5.1	116	29.0	47.4
Stonewall Natural	Tablet	5.1	115	34.0	54.5
Ariva Java	Tablet	1.9	47.5	14.4	19.8
Ariva Wintergreen	Tablet	2.1	47.0	13.9	20.6
	AVERAGE	11.7	192	74.3	248
	MINIMUM	1.9	32	13.9	19.8
	MAXIMUM	28.9	301	133	431

Qualitative Screen

Qualitative Screen: Sample Preparation

- 0.5g ± 0.1g of ground dissolvable product was placed in a 15-mL vial
- 10-mL aliquots of solvent was added (acidic, basic, organics)
- Internal standard solution (MDA) was added.
- Sample was rotated at 60 rpm for 60 minutes.
- Sample extract was filtered to remove suspended solids and then concentrated.
- Concentrated extract was analyzed by GC/MS

Qualitative Screen: GC/MS parameters

- Instrument: Agilent 6890 Gas Chromatograph with 5975 Mass Spectrometer
- Autosampler: LEAP Twin-rail Combi-PAL
- Column: Phenomenex INFERNO column (30m x 0.25mm x 0.25µm)
- GC flow: Helium, 1.1 ml/min
- Inlet Temp: 265°C; splitless mode
- Inlet liner: single taper liner with glass wool
- Injection volume: 1-µl aliquot
- GC ramping program:
- 90°C, hold 2 min; 2°C/min to 325°C; total run=120.5 min.
- MS Full Scan Range: 30 – 800 amu.
- Auxiliary Line: 340°C

GC/MS Qualitative Limitations

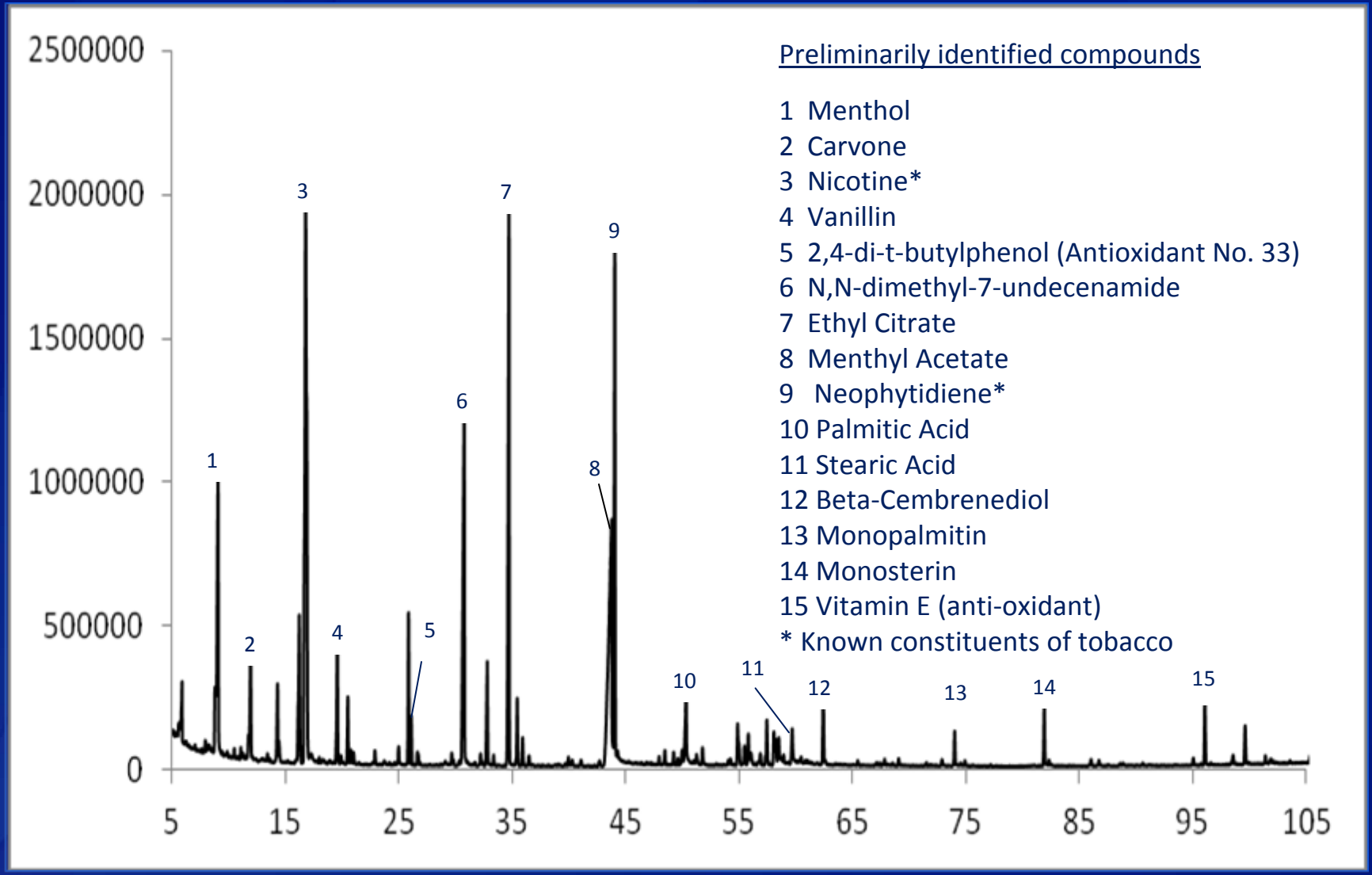
- Approach limited mainly to volatile or semi-volatile components
- Full scan mode provides library searchable data but is not the most sensitive operational mode
- Non-volatile or chemically labile compounds not amenable to this approach
- Limited utility for directly analyzing many harmful or potentially harmful constituents (e.g., heavy metals, heterocyclic amines, aromatic amines, …)

Preliminary Qualitative Identification Parameters

One or more of the following:

- Mass spectral library search (PBM)
- Exact mass analysis
- Known retention times

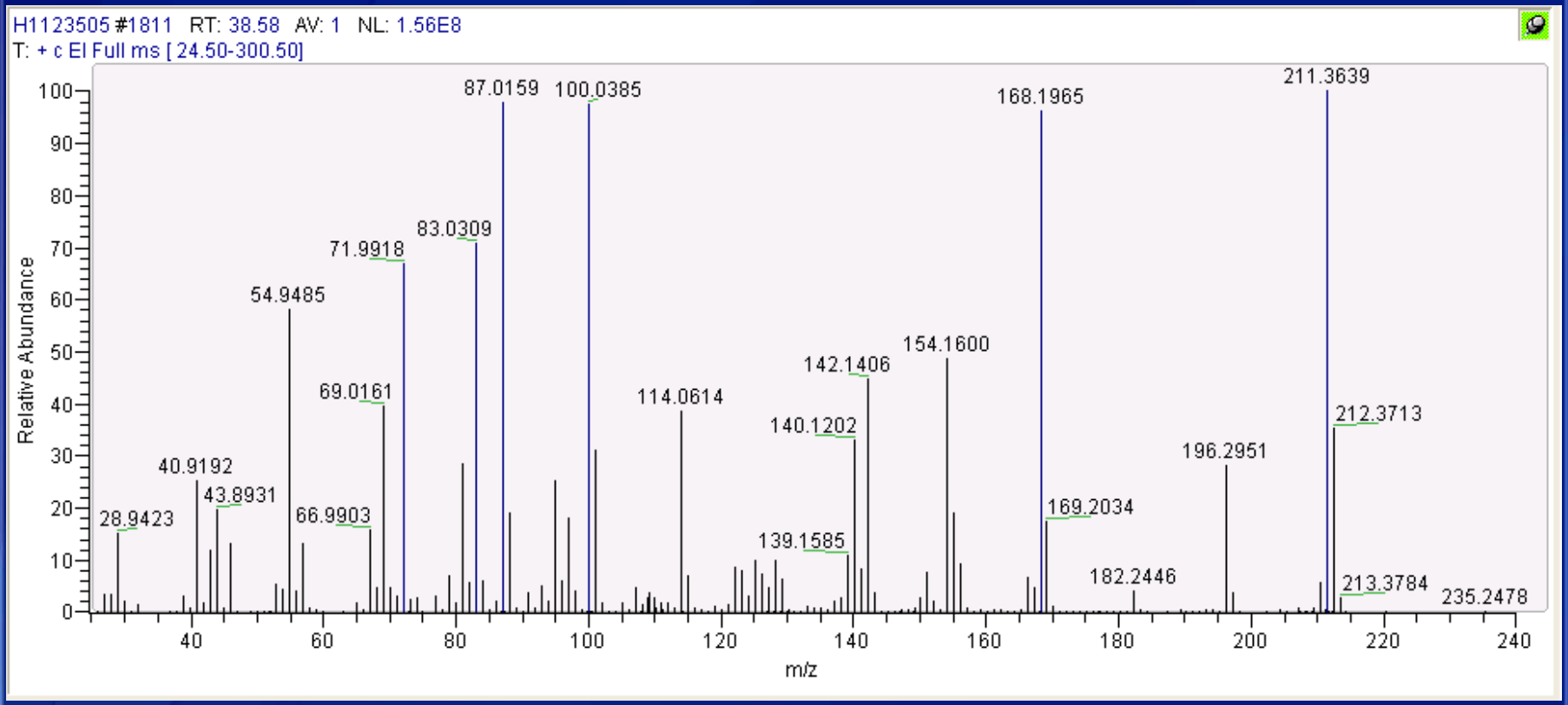
Total Ion Chromatograph of Camel Orbs (Fresh)



Preliminarily identified compounds

1 Menthol
2 Carvone
3 Nicotine*
4 Vanillin
5 2,4-di-t-butylphenol (Antioxidant No. 33)
6 N,N-dimethyl-7-undecenamide
7 Ethyl Citrate
8 Menthyl Acetate
9 Neophytidiene*
10 Palmitic Acid
11 Stearic Acid
12 Beta-Cembrenediol
13 Monopalmitin
14 Monosterin
15 Vitamin E (anti-oxidant)
* Known constituents of tobacco

Preliminary Identification of Unknown Compound using High Resolution Mass Spectrometry



High resolution mass spectral analysis:
Proposed Formula: $C_{13}H_{25}NO$

Preliminary identification : N,N-dimethyl-7-undecenamide (peak 6)

Preliminarily identified flavors and related compounds

3-Octanol
Benzyl Benzoate
Cedr-8-en-15-ol
1-Carvyl Actetate
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
2,6-dimethyl-2,6-octadiene
3-Carene
3-p-Menthene
6-Methylcoumarin
alpha-Humulene
alpha-Methylbutyrolactone
Anethole
Angelica Lactone
Benzyl Benzoate
Benzyl Cinnamate
beta-Caryophyllene
beta-Cubebene
Carvone
Caryophyllene oxide
cis-7-Tetradecenal
cis-Carane
Coumarin
Davanone
Dihydrocareol*
Dihydrocarvone*
D-Limonene
Ethyl Decanoate*
Ethyl Maltol
Ethyl Salicylate
Ethyl Vanillin
Eucalyptol
gamma-Dodecalactone
gamma-Muurolene
gamma-Nonalactone
Germacrene D
Hydrocoumarin
Hydroxymethylfurfurole
Isopulegol
Ledol
Maple Lactone (Corylon)
Menthofuran*
Menthol
Menthone
Menthyl Acetate
Menthyl Lactate*
Methyl Cyclopentenoline
Methyl Salicylate
Methylvanillin
Mint Furanone
Octanolactone
Oxoisophorone
p-Anisaldehyde
Piperitone
Piperonal
Piperonylacetone
Piperonylidene acetone
p-Menth-3-ene
p-Menth-8-en-2-one
p-Menth-8-en-2-one, trans
p-Menth-8-ene
Pulegone
Pyranone
Sabinene Hydrate*
Terpinen-4-ol
trans-beta-Farnasene
trans-Cinnamaldehyde
Vanillin
β-Bourbonene
β-Farnasene
δ-Decalactone

Preliminarily identified flavors and related compounds

Carboxylic Acid
Benzoic Acid

Antioxidants
2,4-Di-tert-butylphenol (Antioxidant 33)
Antioxidant AS
p-Tyrosol [4-(2-Hydroxyethyl)phenol]
Vitamin E Acetate
Vitamin E

Fatty Acids
Hexanoic Acid (Caproic Acid)
Lauric Acid (Dodecanoic Acid)
Myristic Acid (Tetradecanoic Acid)
Palmitic Acid (n-Hexadecanoic Acid)
Stearic acid (Octadecanoic Acid)
alpha-Linolenic Acid (Octadecatrienoic Acid)
Linolic Acid (Octadecadieneoic Acid)

Fatty Alcohols
a-Linolenyl Alcohol
Lauric Alcohol

Amides
N,N-methyl-7-undecenamide†
Oleamide

Humectants
Butoxydiglycol
Diacetin
Triacetin
Glycerin
Glyceryl monoacetate

Tobacco-Related
Nicotine
beta-Nicotyrine
Isonicoteine (2,3'-Dipyridyl)
Geranyl acetone
Nornicotine*
Anatabine*
Myosmine
beta-Cembrenediol
Megastigmatrienone (isomer)
Scopoletin
Cotinine
Neophytidiene
Phytol

Sterols
beta-Sitosterol
Campesterol
Stigmasterol

Ink Solvent
Butoxyethyl Ethanol

Plastisizer
Ethyl Citrate

Polymer residue
2-Pyrrolidinone

Acrylate
Lauryl Acrylate

Hydrocarbon
Squalene

Fatty Acid Esters
Ethyl Laurinate
Ethyl Palmitate
Ethyl Stearate
Methyl Palmitate
Methyl Stearate
2-Monostearin
2-Monopalmitin

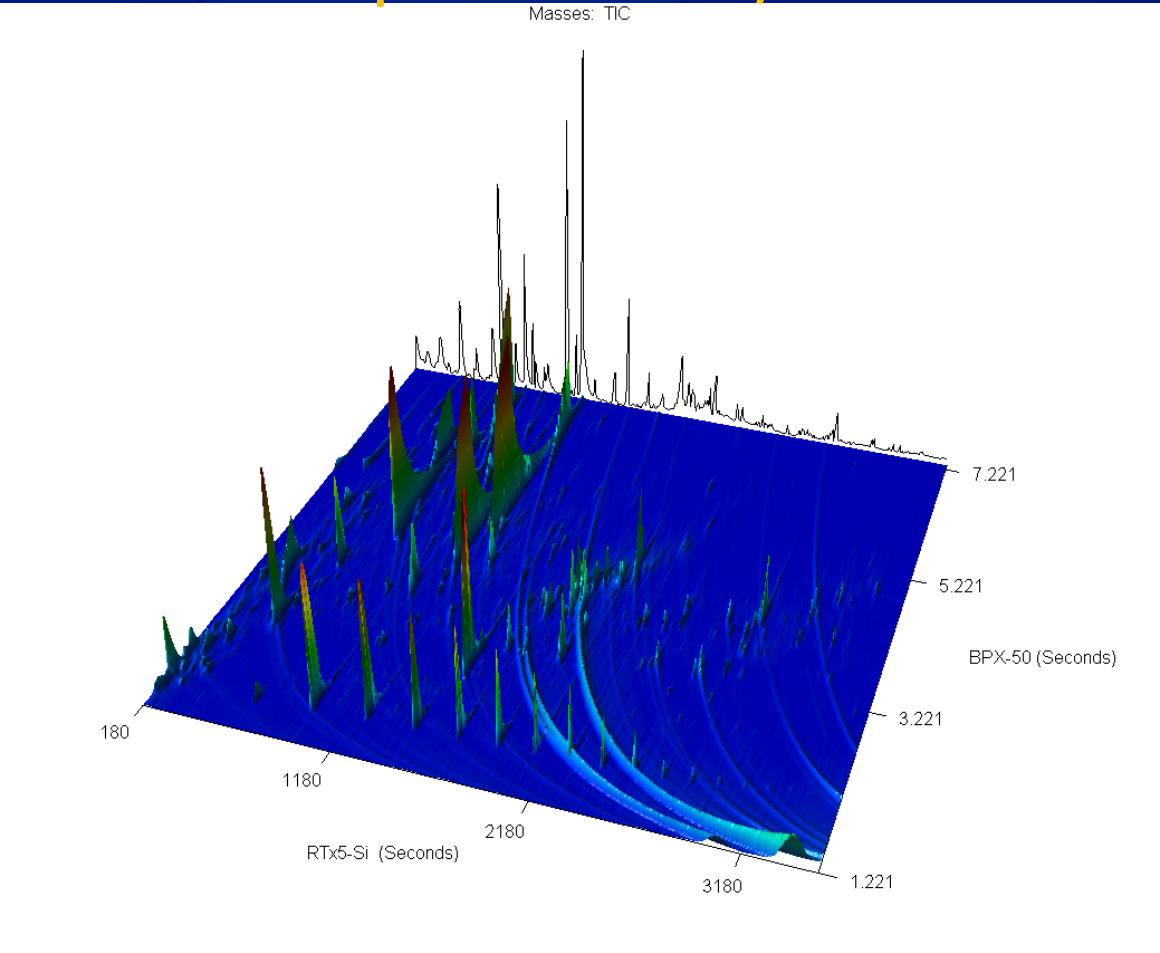
Preservatives
Methylparaben
Propylparaben (preservative)

Preliminary Identification of Other Compounds Using Two-Dimensional Gas Chromatography/Time-of-Flight Mass Spectrometry

Qualitative Screen: GCxGC/TOF-MS parameters

- Column First Dimension: Restek Rxi5MS (20m x 0.18mm x 0.18µm)
- Column Second Dimension: BPX50 (1m x 0.10mm x 0.10µm)
- GC flow: Helium, 0.6 ml/min
- Inlet Temp: 275°C; splitless mode
- Inlet liner: single taper liner with glass wool
- Injection volume: 1-µl aliquot
- GC ramping program (first dimension):
- 90°C, hold 1 min; 4°C/min to 325°C; hold 4 min
- GC ramping program (second dimension):
- 95°C, hold 1 min; 4°C/min to 330°C; hold 4 min
- MS Full Scan Range: 35-450 amu
- Acquisition rate: 150 spectra/second
- Auxiliary Line: 350°C

Example: GCxGC/TOF-MS



- >1700 spectral hits
- 163 compounds preliminarily identified (vs 32 for GC/MS)

Compounds preliminarily identified* (GCxGC)

Phthalate compounds (6)
Diethyl Phthalate
Diisobutyl Phthalate
N-Butyl Phthalate
Diisooctyl phthalate
bis(7-methyloctyl) phthalate
o-Phthalic Acid

Lactones
Crotonolactone
1,4-Butyrolactone
gamma-Dodecalactone

Furans
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
2-Hydroxyacetylfuran
2(5H)-Furanone, 5-(2-hydroxyethylidene)-
Dibenzofuran P154

Sterols/Vitamins
Cholestra-3,5-diene
gamma-Tocopherol (Vitamin E)
Cholest-4-en-6-one
Cholesterol
Vitamin K

Tobacco
4-vinylphenol (4-Hydroxystryene)
Ethylmethylmaleimide
Nicotine
(E)-Solonone
Myosmine
Geranyl Acetone
Nicotine, 1'-Oxide
alpha-Nicotyrine
Isonicoteine
Megastigmatrienone (Isomer 2)
3-Hydroxy-alpha-damascone
3-oxo-alpha-ionol
Cotinine
Loliolide
Neophytadiene
Occidol
Farnesyl acetone
Cembrene
Isophytol
Scopoletin
Sclareolide
beta-Cembrenediol

Fatty Acids
3-Methyl-Butanoic Acid
2-Methylbutanoic acid
Pentanoic Acid
Caproic Acid
Heptanoic acid
Octanoic Acid (Caprylic acid)
Leinoleic acid

Phenolic Compounds
Phenol
p-Cresol
4-Nonylphenol
2,6-Di-tert-butylhydroquinone

Chlorinated compounds
3-Pyridyl Cyanide (Chloromethylbenzene)
Chlorotoluene
2-Methoxy-4-Chlorophenol

Herbicide
Herboxide

Antioxidants
2,4-Di-tert-butylphenol (Antioxidant No 33)
Tyrosol

*** Camel Orb Fresh**

Flavor related compounds preliminarily identified* (GCxGC)

5-Hexen-2-one
2-Acetyl Furan
1,2-Cyclopentanedione
alpha-Angelica lactone
6-Methyl-5-hepten-2-one
Isomaltol
2,4-Heptadien-1-al
Isonicotinic aldehyde
2H-Pyran-2-one (Coumalin)
Cymene
Limonene
Benzyl Alcohol
(6E)-2,6-Dimethyl-2,6-octadiene
Pineapple ketone
d-Camphene
Maltol
Citral

N-Methylsuccinimide
alpha-Phellandrene
2-Phenethyl Alcohol
4-Oxoisophorone
Pyranone
cis-Menthone
Phenyl Ethyl Ketone (FEMA 3469)
4-Hydroxydihydro-2(3H)-furanone
Menthol
4'-Methylacetophenone
Terpenol
Dihydrocarveol
3'-Methylacetophenone
3-Acetyl Pyridine
trans-Carveol
Cis-Carveol
Carvone
D-Piperitone

gamma-Octalactone
Menthyl acetate
2,4-Decadienal
cis-Dihydrocarvyl Acetate
(-)-trans-Carveol Acetate
gamma-Nonalactone (Coconut aldehyde)
(-)-alpha-Bourbonene
Jasmone
Vanillin
alpha-Terpineol
alpha-Farnesene
Acetovanillone
Viridiflorol
Ethyl Citrate
Cedr-8-en-15-ol
alpha-Undecalactone
Benzophenone

Summary

- GC/MS and GCxGC/TOF-MS provided qualitative information on many volatile and semi-volatile constituents
- Dissolvable tobacco products are chemically complex
- Current approach not applicable to thermally labile compounds
- More work would be required to confirm the tentative identifications and indentify the remaining constituents.

Acknowledgment

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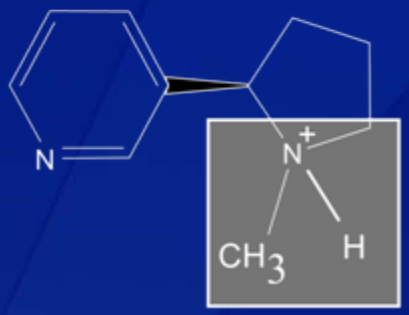
Bryce Duncan
Steve Pappas

Supplemental Information

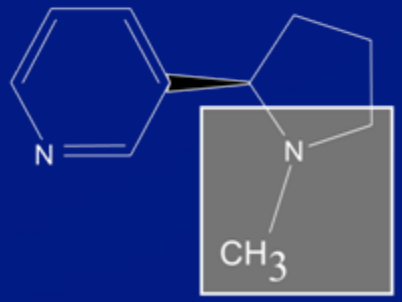
The form of nicotine affects how fast it gets to the brain (faster = more addictive)

Nicotine



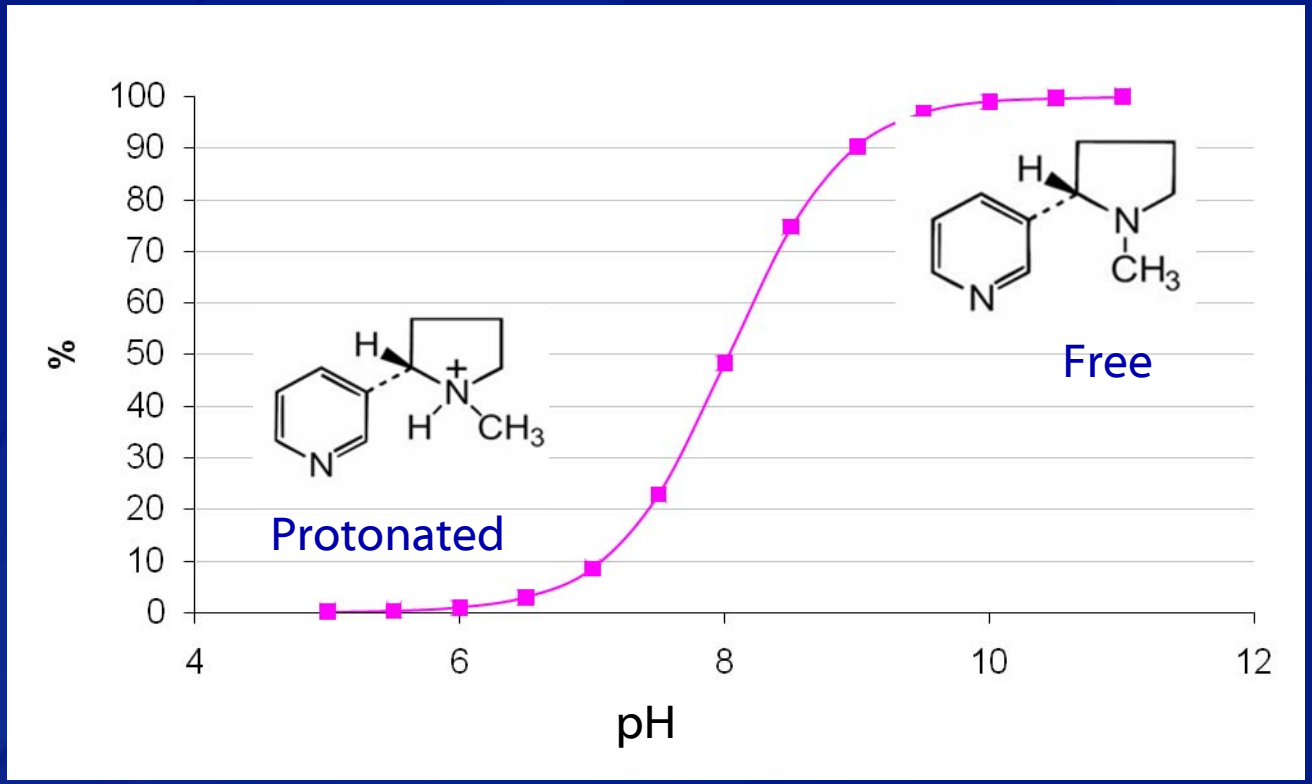
base

Free nicotine

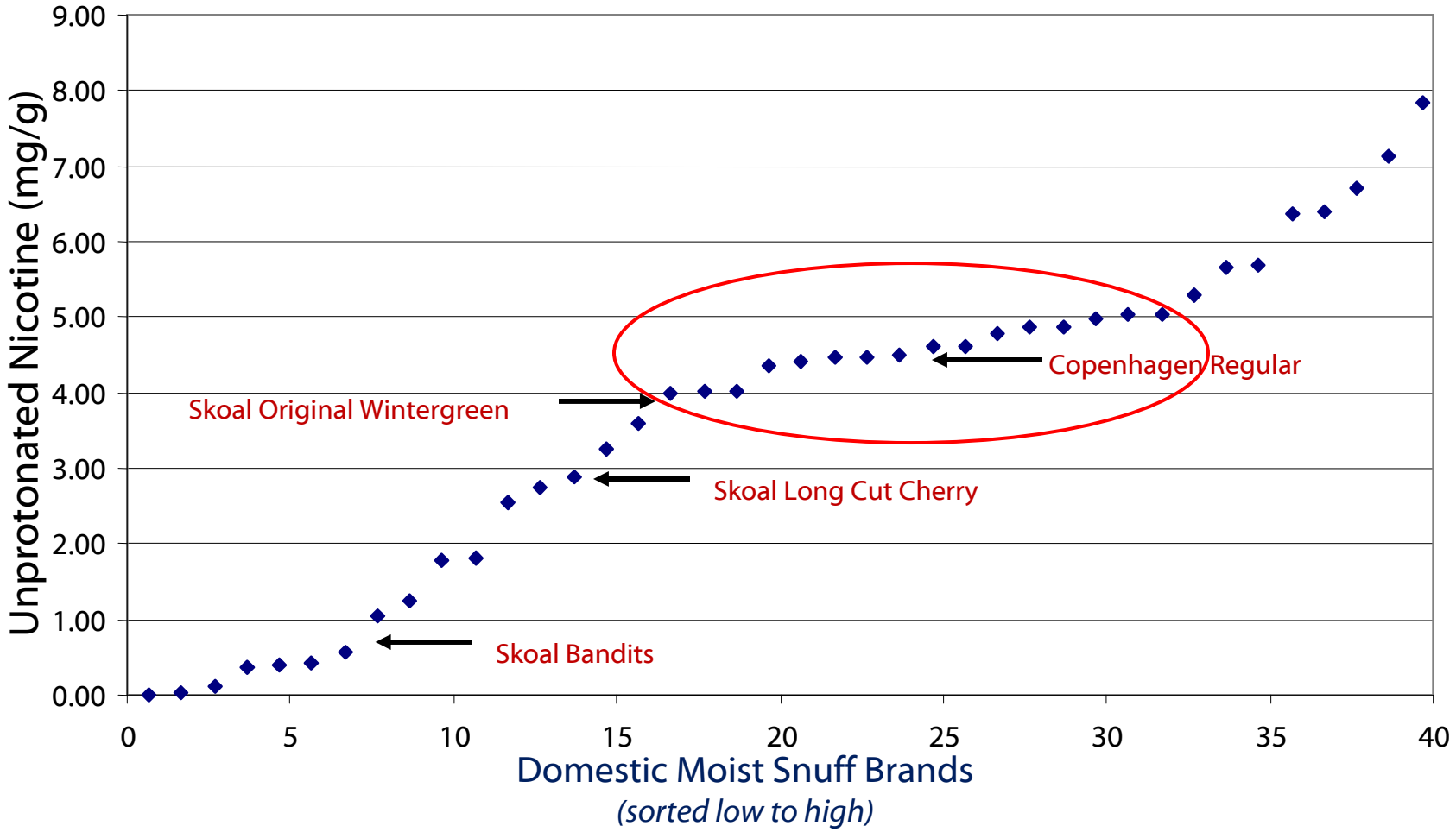


Absorbs faster

Acid/base properties of nicotine

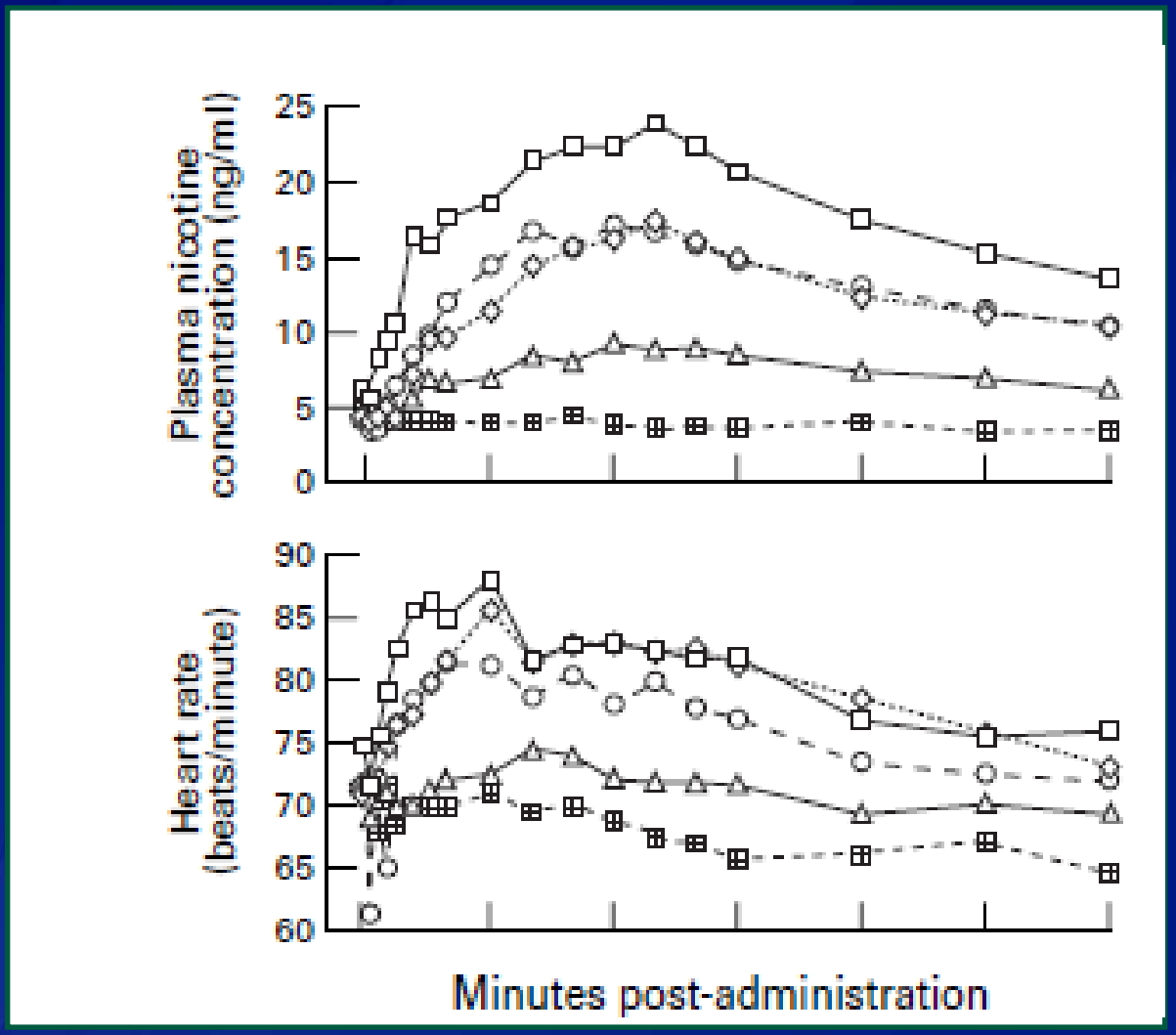


Unprotonated nicotine levels



Richter, P; Hodge, K; Stanfill, S; Zhang, L; Watson, C (2008) "Surveillance of moist snuff: total nicotine, moisture, pH, un-ionized nicotine, and tobacco-specific nitrosamines", Nicotine and Tobacco Research 10: 1645-1652.

Nicotine uptake and heart rate data from moist snuff products



•Reginald V Fant, Jack E Henningfield, Richard A Nelson, Wallace B Pickworth (1999) *Tobacco Control 1999;8:387–392.*