I STEEP MY TEA, SO DO I NEED TO STEEP MY E-LIQUIDS?

John H. Lauterbach, Ph.D., DABT

Lauterbach & Associates, LLC, Macon, GA 31210 USA

Steeping – science, folklore or both?

- For tea, there is considerable scientific evidence for chemical changes occurring during steeping; for example
  - Wang et al., *JAOAC Intl*., 2019 Feb 22. Effect of different steeping temperatures on leaching of aroma components in black tea…
  - Liu et al., *Food Chem*. 2017 234:168-173. Effect of steeping temperature on antioxidant and inhibitory activities of green tea extracts against α-amylase, α-glucosidase, and intestinal glucose uptake
  - Hajiaghaalipour et al., J Food Sci. 2016 81:H246-54. Temperature and time of steeping affect the antioxidant properties of white, green, and black tea infusions
- Tea is a complex natural product, but contemporary e-liquids can also be complex and may contain ingredients of natural origin and variable composition such as flavors (*i.e.*, menthol)

For e-liquids, steeping appears to be folklore

- Articles and videos on the Internet tell you why and how to steep, but is information technically correct? Some examples
  - Steeping vape juice is a way of aging the e-juice to remove any harsh bottled tastes and alcohol, which are volatile
  - Steeping mixes the VG/PG base, flavorings, and nicotine, and then oxidizes the juice to remove the alcohol. The process of oxidation, while removing the alcohol, also darkens the color of the liquid
  - Steeping will make the juice have a stronger, but not harsh, and more flavorful taste and smell to it. By steeping your e-liquid, it can produce an improved throat hit. Certain e-liquids may even produce more clouds than they did before the e-liquid was steeped
- Apparently, no scientific evidence (*e.g.*, chemistry, sensory panel data, etc.) available to justify such statements

How do you steep your e-liquids?

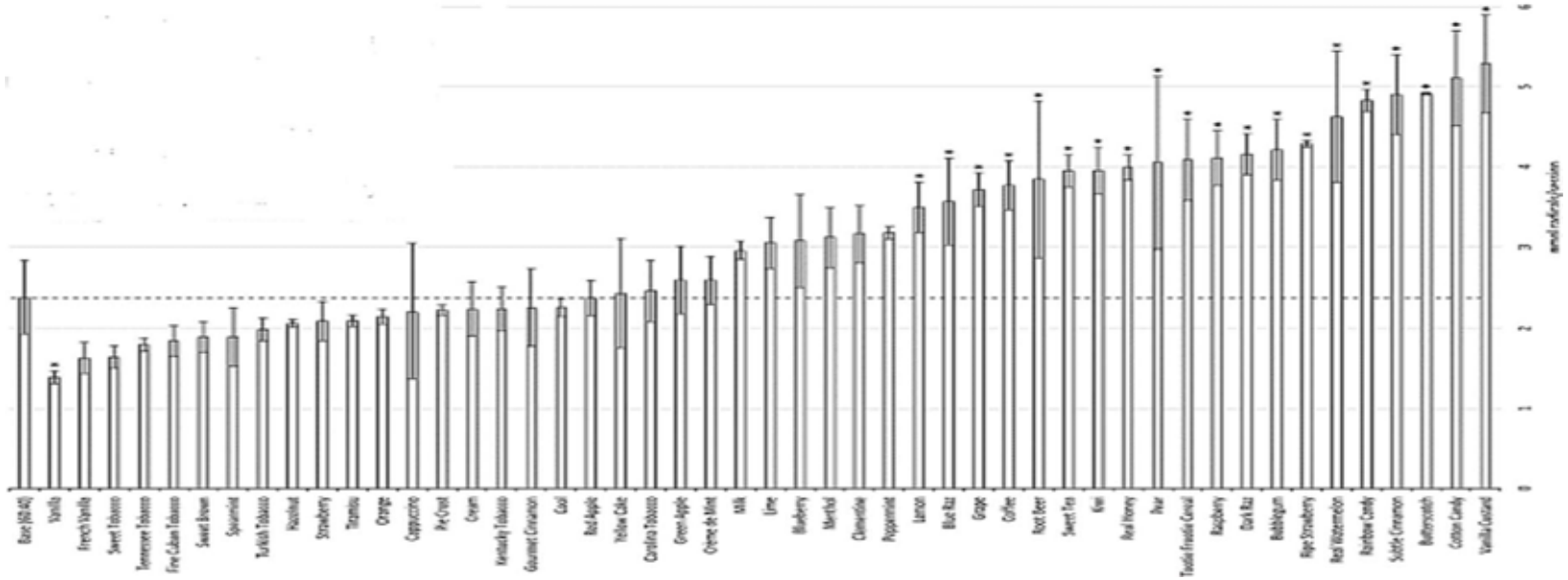
- Four YouTube videos provide instructions and some results
  - "STEEPING your E-juice! Why you need to be doing this." https://www.youtube.com/watch?v=fP5YKiawHU8
  - "Steeping Methods For Your E-Liquid." https://www.youtube.com/watch?v=BkOE-blQBtc
  - "How to Steep your E-Liquid – Zamplebox Vape School." https://www.youtube.com/watch?v=dUyuln2eU-4
  - "Ultrasonic Steeping For E-Liquid?" https://www.youtube.com/watch?v=6R068nmHyF8
- Several ways of steeping
  - Keeping in dark for 8 weeks with occasional shaking with/without opening bottles to allow e-liquids to "breath"
  - Warm-water baths or ultrasonic cleaners with water baths

Does steeping change the chemistry?

- Many reports that steeping changes the taste of the aerosol
  - Are changes caused by better mixing of the e-liquid components
  - Does “breathing” allow off-taste components to escape
  - Are chemical changes responsible for change in aerosol taste
  - Are chemical changes only minor or are major components involved?
  - Are HPHC or precursors formed
- Some chemical changes expected
  - Reaction products (acetals) of aromatic aldehydes with PG known
  - Some are commercial products such as PG acetals of vanillin, ethyl vanillin, and benzaldehyde
  - Similar VG acetals reported as well as L-menthone 1,2 glycerol ketal
- Are slow selling products changing chemically while they are on store shelves as that may be like steeping for 8 weeks

Is inadvertent steeping a problem?

- Journal articles have reported unexpected e-liquid toxicity
  - Unexpected toxicity in *in vitro* cytotoxicity assays, and toxicants as reported by Bitzer et al., Free Radic Biol Med. 2018 120:72-79. Effect of flavoring chemicals on free radical formation in e-cig aerosols



Did inadvertent steeping change Bitzer's results?

- Bitzer's procedures
  - Commercial flavor concentrates (in PG) were diluted with PG and VG for final PG/VG of 60/40 with 20% flavor-concentrate concentration
  - No nicotine was added; no data on time from mixing to analysis
  - E-liquids vaporized in Wismec Reuleaux RX200S Mod operating at 225°C and 50 W with 0.5 Ω SS coil using 8.3/5/30 puffing regimen
- Some of Bitzer's results
  - E-liquids with ethyl maltol and piperonal gave aerosol with higher free radical concentrations than most other constituents
  - Ethyl vanillin, its PG acetal δ-tetradecalactone tended to reduce free radical concentration, but γ-decalactone had the opposite effect
- Did Bitzer miss something by not adding nicotine – possibly as his e-liquids could have been acidic

A new approach to understand steeping

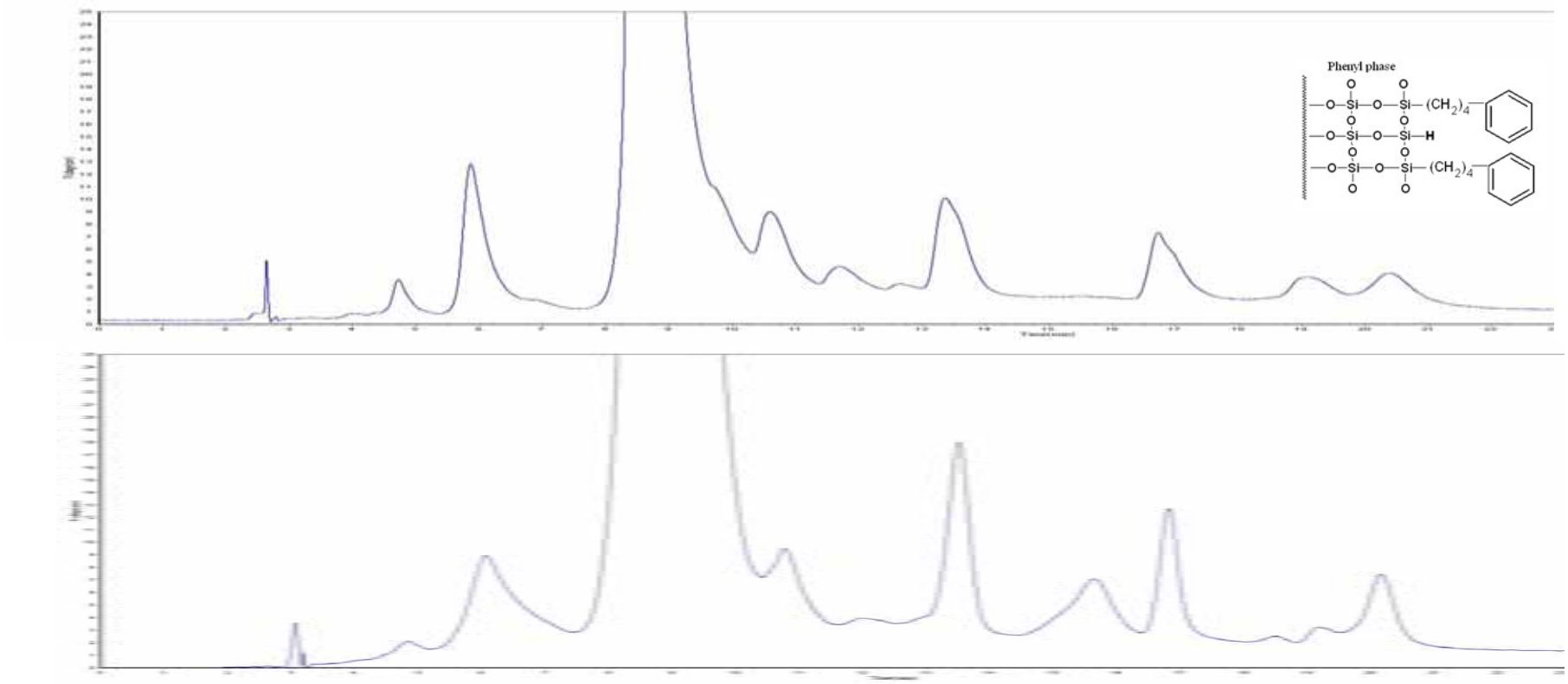
- Formulating e-liquids with nicotine
  - Commercial flavor (14 different ones used) concentrate (in PG) 4 mL
  - 50/50 VG/PG mixture 6 mL
  - 100 mg/mL nicotine in VG 4 mL
  - Estimated nicotine 28.6 mg/mL, PG 7 mL, VG 7 mL
  - Alternate formulas used 2 mL concentrate, 8 mL all PG or all VG, 4 mL 100 mg/mL in PG or in VG
  - Samples placed in 1-oz screwcap jars
- Steeping
  - 70-W DSA ultrasonic cleaner with water bath and sample basket
  - Four 30-minute treatments separated by 30-minute cooling periods with ultrasonic cleaner turned off
  - Bath temperature and e-liquid temperatures exceeded 70° at times

Did steeping change the e-liquids? Yes and no

- Analysis of unsteeped/steeped e-liquids by HPLC
  - Many would use GC, but looking for major changes, not minor ones
  - In-house instrumentation allowed samples to be analyzed under several very different conditions
  - Instrumentation (all Waters) three 510 pumps, 680 gradient controller, 486 tunable absorbance detector; 410 refractive index detector, U6K injector; Rheodyne 7725i injector and two Surwit N2000 dual channel chromatography data systems
- HPLC columns (250 mm X 4.6 mm) and mobile phases used
  - Cogent Type-C silica, Amide, Bidentate C18, Phenyl Hydride
  - YMC Triart C18
  - Higgins Analytical, CLIPEUS C18
  - Cogent columns used with ACN/H2O and EtOH/H2O; other columns used with MeOH/H2O

Chromatograms – Sweet, fruity e-liquid

Steeped vs nonsteeped, Phenyl Hydride, 280 nm, 38/62 EtOH/H2O to 67/33 EtOH/H2O RP



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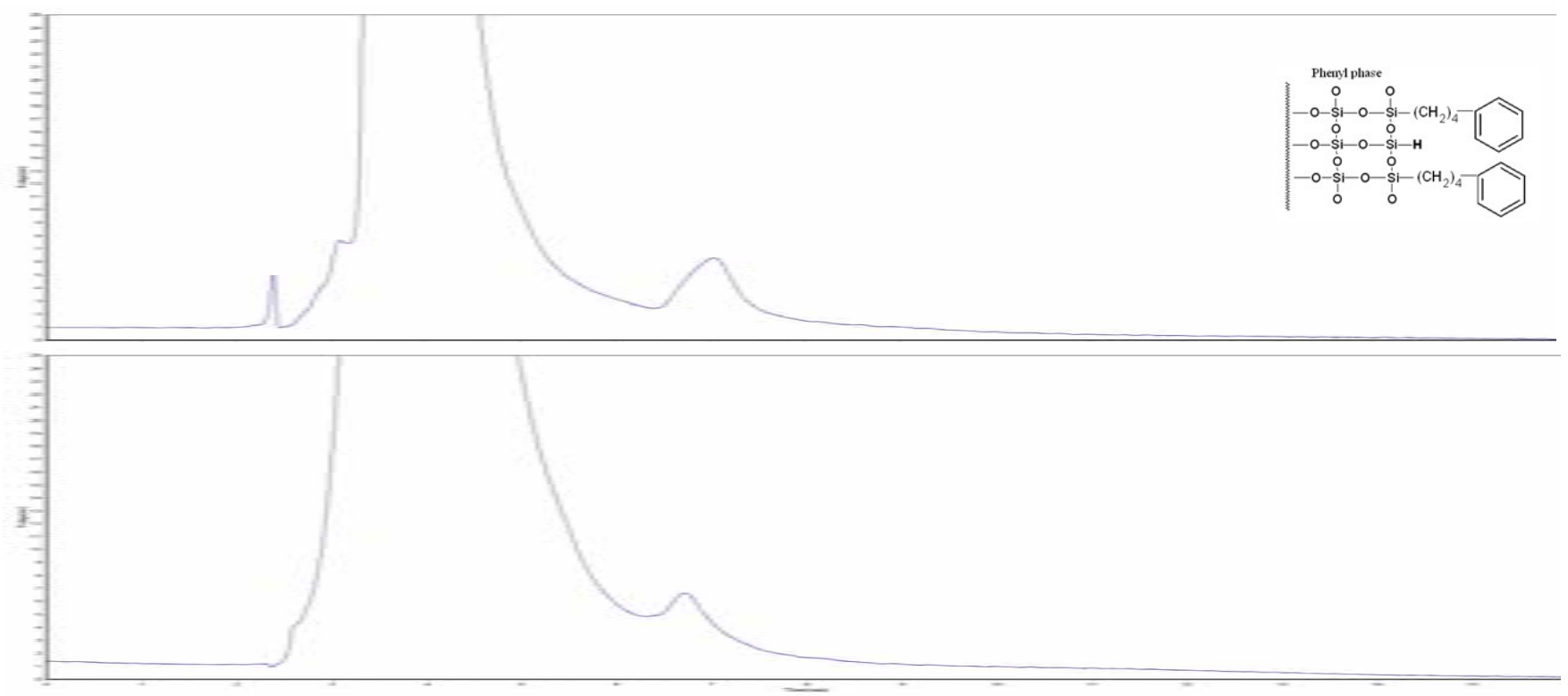
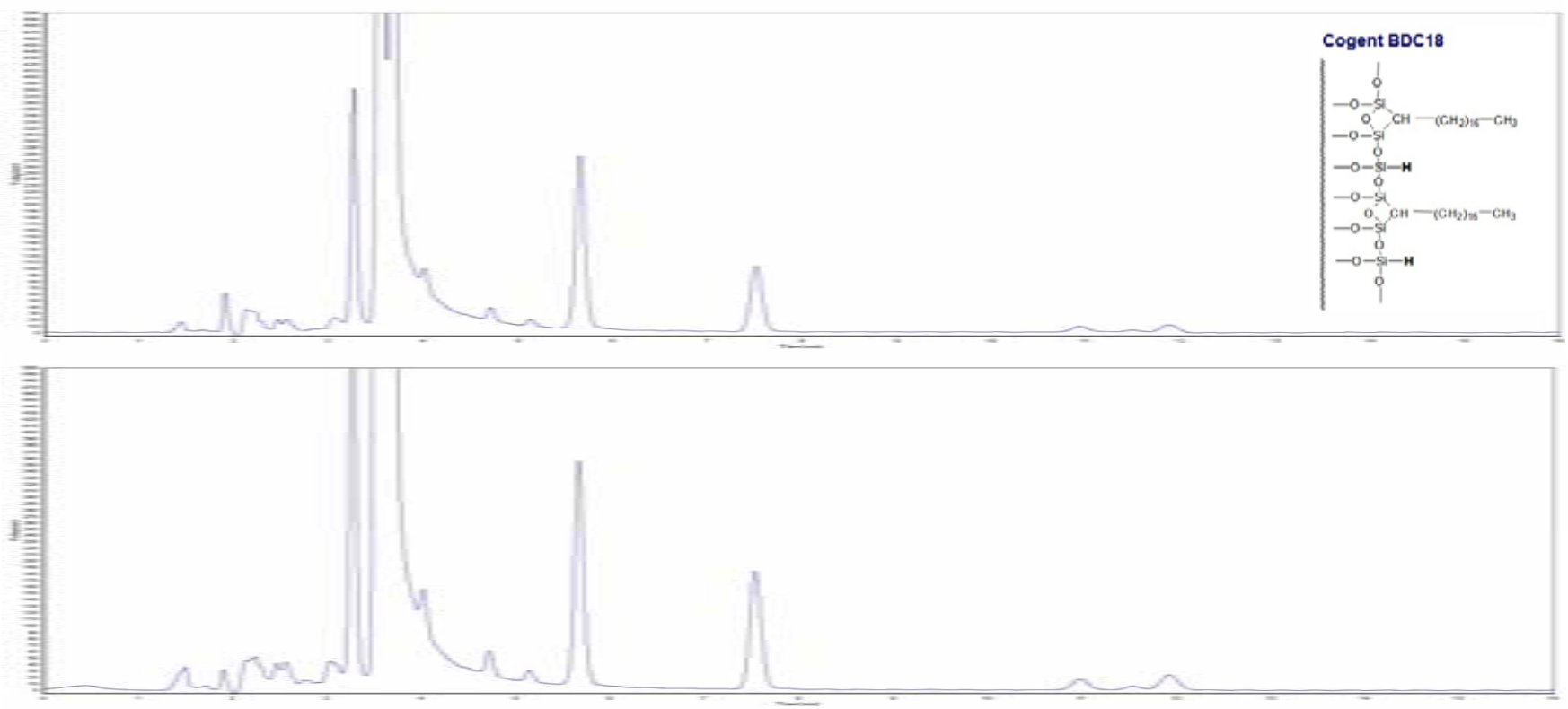


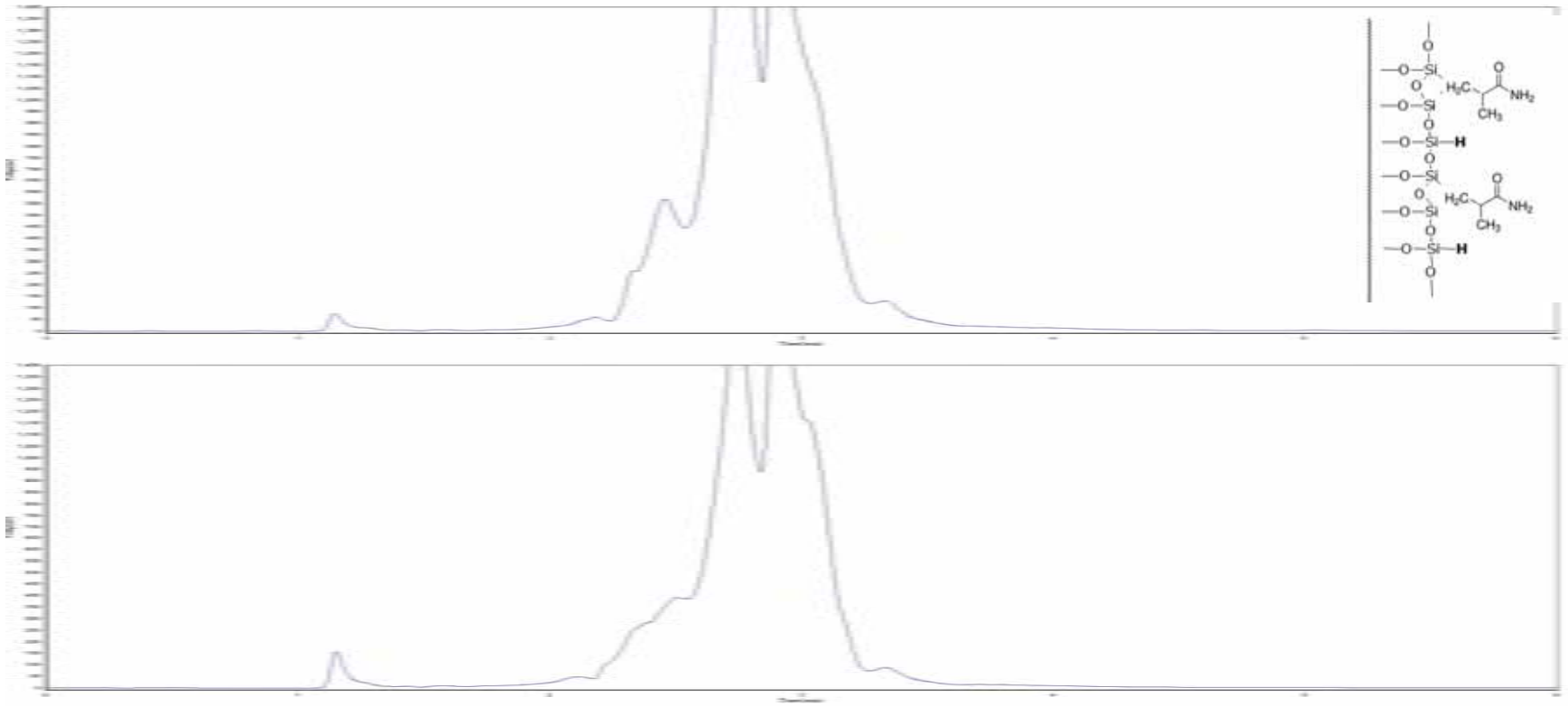
Chromatograms – Sweet, fruity e-liquid

Steeped vs nonsteeped, Bidentate C18 195 nm, 50/50 ACN/H2O ANP



Chromatograms – Custard with vanilla

Steeped PG vs steeped VG, Amide 195 nm, 50/50 ACN/H2O ANP



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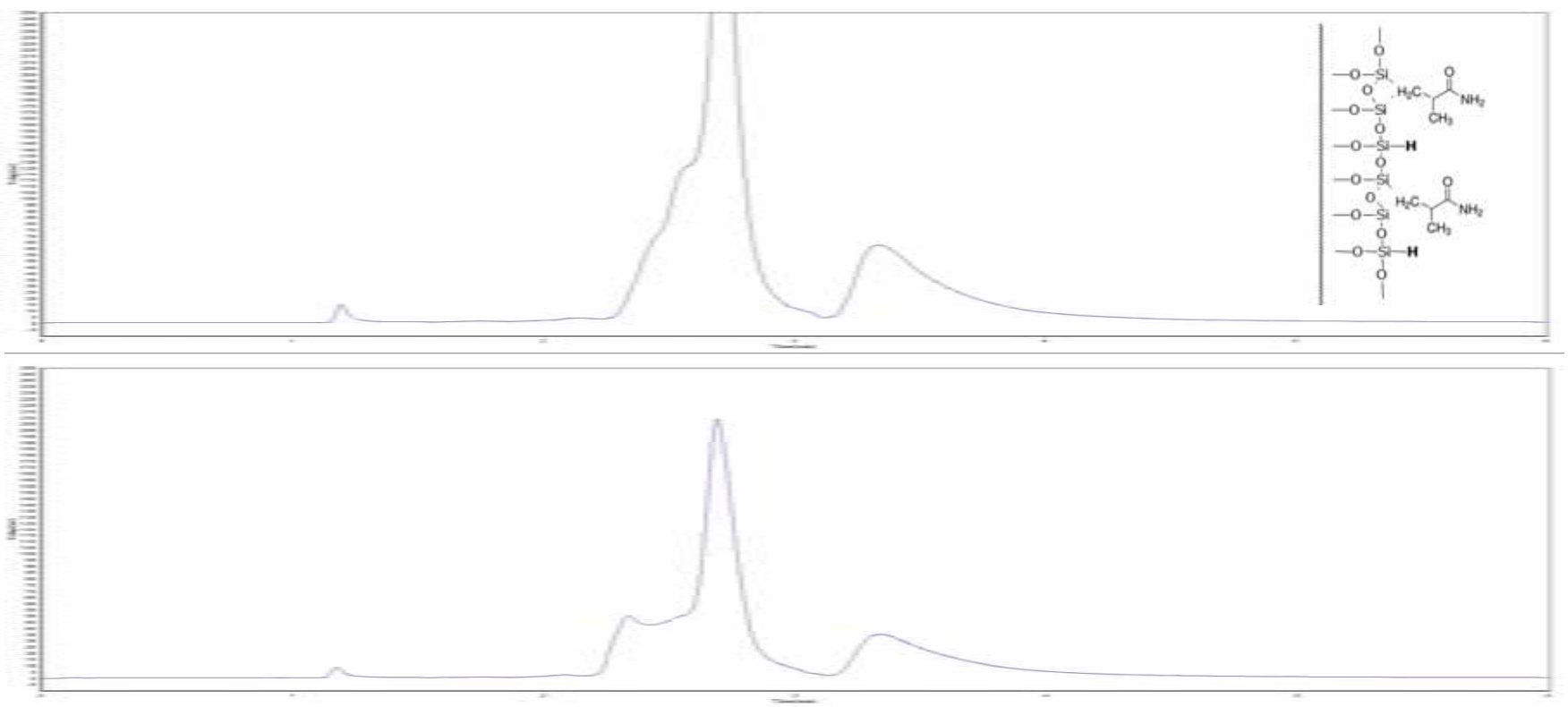

Conclusions

- Steeping
  - Steeping can change the compositions of e-liquids
  - While only a few examples shown, steeping effects more pronounced for e-liquids containing aromatic aldehydes as flavorings
  - Analyses of simple e-liquids (*e.g.*, V2 Red 2.4 and V2 Menthol 2.4) over a several-year period did not show changes
  - Analyses of many e-liquids similar to those used by Bitzer showed that complexity of the compositions was related to his results
  - Inadvertent steeping needs to be avoided when analyzing e-liquids
- Chromatography
  - HPLC with use of several different chromatography conditions (*e.g.*, column, mobile phase, and detector wavelength) can be used to monitor changes that e-liquids undergo during steeping