You can't analyze for everything or can you?

J.H. Lauterbach, Ph.D., DABT
Lauterbach & Associates, LLC, Macon, GA 31210-4708 USA

Abstract
Some regulators only want data on a product's emissions and/or certain constituents in the tobacco or e-liquids [e.g., US FDA Harmful and Potentially Harmful Constituents (HPHC)]. Other regulators want information of new constituents formed during processing (reaction of reducing sugars with amino acids and proteins in the tobacco) or storage/shipment (formation of acetals during in an e-liquid containing aromatic aldehydes and propylene glycol). Regulators have also called for data to show that products have been manufactured correctly. Numerous techniques and methods have been reported in the literature, conference proceedings, and legacy documents that can be used to provide data to the regulators. However, many of the techniques and methods require complex instrumentation and highly-trained laboratory personnel such as found at the major tobacco and e-vapor companies and commercial laboratories. Consequently, something simpler is needed. One approach is liquid chromatography (aka LC, HPLC), but not with the column technology used in the past (e.g., methods for casings on tobacco). The new technology involves the so-called Type-C silica and permits the columns to be used in both the traditional reverse-phase (RP) and new aqueous-mobile-phase (ANP) modes. Thus, samples of e-liquids or tobaccos, diluted with or extracted with 50/50 (*v*/*v*) acetonitrile/water (or similar solvents) can be chromatographed under normal RP conditions and alternate separations to resolve coeluting peaks performed under ANP conditions. This can be done without changing columns. Examples will be provided using a Cogent™ Phenyl Hydride column and a YMC™ Triart C18 column with gradients of water-acetonitrile for RP separations and acetonitrile-water for ANP separations and will be provided for complex e-liquids, heavily processed commercial tobacco products (e.g., pipe tobaccos) and artificial salivas exposed to e-cigarette aerosols.

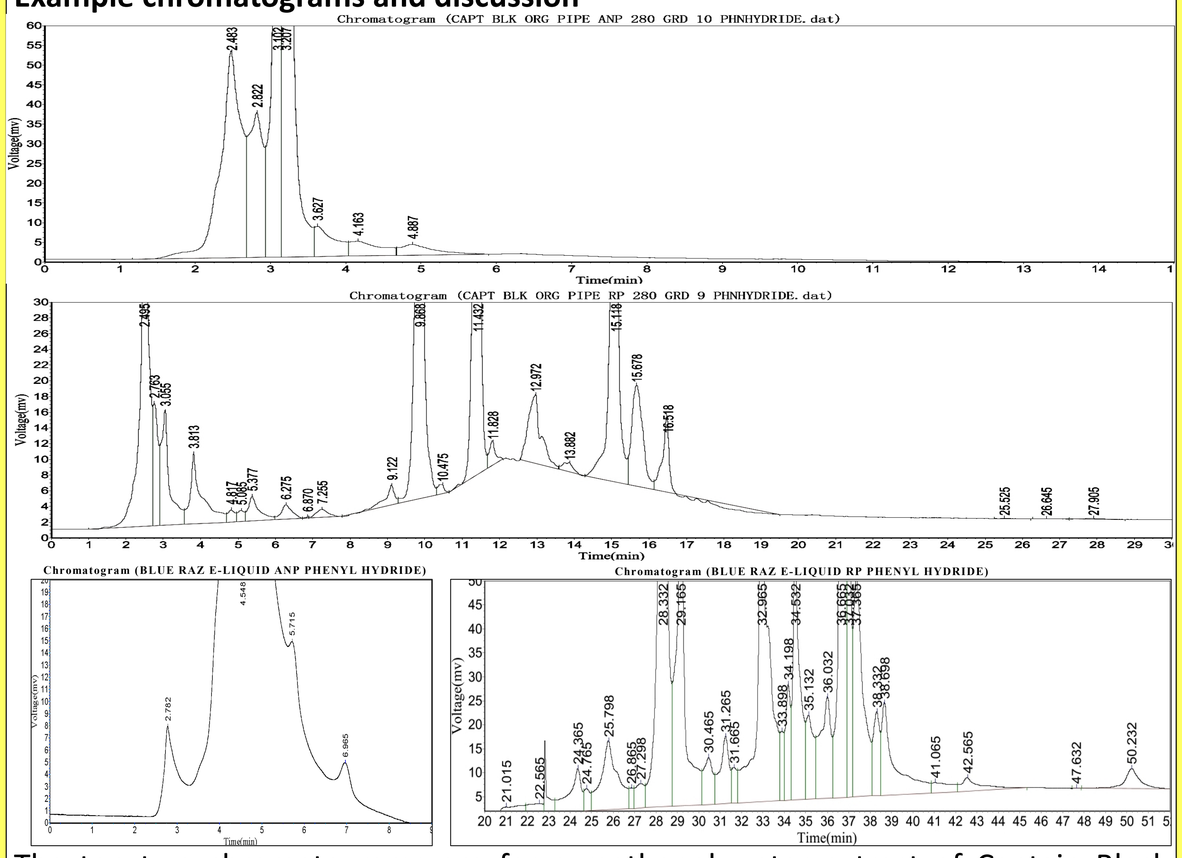
Introduction
Industrial analytical laboratories exist for three purposes: 1) to make sure that their company's products are manufactured to specifications; 2) to find out how competitors made their products; and 3) to provide information to make their company's more competitive. For companies that must submit data to regulators, they generally need to have such data generated by accredited in-house laboratories or pay accredited commercial testing laboratories to generate the needed data. Small manufacturers often do not have the resources needed to have their own accredited laboratories especially when the data required by regulators must be generated with expensive instrumentation that requires skilled operators. However, use of commercial testing laboratories can be very costly so that it is essential that products sent for testing were manufactured correctly. When chemical assays are needed, liquid chromatography (aka LC, HPLC) if often the best choice. However, LC can be costly and complex. Consequently, the purpose of this presentation is to show how cost and complexity can be reduced through judicious choices of instrumentation and operating conditions.

Experimental conditions
LC systems were based on Waters 501/510 pumps, 680 gradient controller, U6K or Rheodyne 7725i injector, 486 tunable absorbance detectors, and Surwit N2000 chromatography 2-channel data system and an HP 3396 Series III Integrator. 10-µL injection volumes were used. Three LC columns were used in the experimental work: 1) Cogent™ Phenyl Hydride - 4µm 100Å – 250mm x 4.6 mm ID; 2) YMC-Triart C18 - 5µm 120Å – 250mm x 4.6 mm ID; and 3) Higgins Analytical CLIPEUS C18 - 10 µm 120Å – 250mm x 4.6 mm ID. Mobile phase was a mixture of water and acetonitrile (ACN) as described in each chromatogram. Samples were diluted with ACN or extracted with ACN/water or methanol/water (MeOH/H2O).

Discussion of LC column packings
Three types of LC columns were used in this study. Two of them represent contemporary reverse-phase (RP) column technology while the third (Higgins) represents older technology that was typical of column technology used around 1980 when LC methods became popular in tobacco analytical laboratories. RP methods for determining the levels of cocoa and licorice added as casing ingredients and estimation of blend composition using polyphenols levels in the flue-cured, burley, and oriental tobaccos and determination of vanillin and related compounds pipe tobaccos. More LC techniques were added when information was needed on harmful and potentially harmful components (HPHC, some formerly known as Hoffmann analytes) in tobacco, tobacco smoke, and more recently, e-liquids, and aerosols produced from those e-liquids. Much of the improvement in RP column technology has been driven by needs of the biomedical industries and part of that has dealt with eliminating or minimizing the adverse effects of the free silanol groups (Si-OH) on the chromatography including irreversible binding of the analytes to the silica. According to the manufacturer's literature, the YMC Triart technology involves blocking the free silanol groups with so-called end-capping groups. This is claimed by the manufacturer is that it tends to make the surface of the silica more hydrophobic [YMC, YMC_GC. 2015 12(4):49-69]. Considerably more literature is available on Type-C silica columns [Pesek et al., J Sep Sci. 2013 Sep;36(17):2760-6 2013]. Bonds on surface of silica are Si-H and Si-O-Si-R (R=phenyl or C18). In this study, the Cogent™ Phenyl Hydride column was used as it was reported to be preferred for aromatic compounds and was known to work with polyphenols [Young et al., J Sep Sci. 2017 Apr;40(7):1449-1456].

Aqueous Normal Phase (ANP) versus Reverse Phase (RP)
Most of the LC work associated with tobacco and e-vapor products has been done with RP columns that were developed for relatively nonpolar compounds. However, analytes such as tobacco polyphenols and acetals of aromatic aldehydes (reaction products that can be found in vanilla-, cherry-, and cinnamon-flavored e-liquids) are quite polar and likely more amenable to so-called ANP chromatography on certain columns that can be used for RP work. Gradient elution is used with both ANP and RP. In RP, the mobile phase goes from polar to less polar (e.g., 90 H2O/10 MeOH to 10 H2O/90 MeOH). This often means that analytes of interest elute in the middle to the end of the LC run instead of near the start of the run, which would save time. In ANP with Type C silica columns such as the Cogent Phenyl Hydride column where acetonitrile (ACN) is used instead of MeOH, the mobile phase goes from less polar to more polar (e.g., 20 H2O/80 ACN to 80 H2O/20 ACN). Several examples from current research projects at Lauterbach & Associates, LLC, were used to evaluate ANP and RP technologies. These included MeOH/H2O extracts of pipe tobacco, e-liquids and components, and artificial salivas that had been exposed to aerosols generated by e-cigarettes. ANP mobile-phase and gradient selection was based on the Cogent™ Quick Method Development Strategy for Cogent™ TYPE-C™ Silica Hydride Based, Bonded Stationary Phases (https://mtc-usa.com/PDF/TypeCQuickStart.pdf). Relevant journal articles are Young et al., J Sep Sci. 2017 Apr;40(7):1449-1456 (polyphenols in fruit); Kulsing et al., J Phys Chem B. 2015 Feb 19;119(7):3063-69 (silicon hydride selectivity); Pesek et al., Anal. Methods 2014 6:4496-4503 (review); Young et al., J Liq Chromatogr Relat Technol. 2013 Apr 1;36(7):926-942 (phenyl hydride); Pesek et al., (aqueous normal phase) Trends Analyt Chem. 2013 42:64-73; and Matyska and Pesek. LC-GC North America. 2007 25(5):480-490 (HILIC vs. ANP).

Example chromatograms and discussion



The top two chromatograms are for a methanol water extract of Captain Black Original brand pipe tobacco. The Cogent Phenyl Hydride column was used with UV detection at 280 nm. The major analytes in that extract are chlorogenic acid and its isomers, rutin, and scopoletin. When the analyses are done with detection at 340 nm, the results are specific for the tobacco polyphenols. Thus, significant time can be saved by using ANP versus traditional RP conditions as shown in chromatogram below the ANP one. Note that the same reagents and instrumentation are used for both ANP and RB. The only difference in the ratio of ACN to H2O in the mobile phase. The two chromatograms on the bottom row are for a diluted (ACN) e-liquid that was formulated with 2g of a flavor concentrate formulated in propylene glycol (PG) and then further diluted with 4g PG and 4g glycerol. The ANP chromatogram on the left is dominated by suspected PG acetals of the aromatic aldehydes such as vanillin, ethyl vanillin and piperonal. Thermal decomposition of those may contribute to the levels of formaldehyde and acetaldehydes found in aerosols generated from e-liquids. Again, use of ANP permits a quick look at the acetals without using RP conditions as shown in the chromatogram on the right.

Conclusions
The space limitations of this poster prevented the display of chromatograms generated with the other two columns. While some useful data were obtained with the YMC™ Triart-C18 column for polyphenols when operated under similar gradient elution conditions to those used with the Cogent™ Phenyl Hydride column, it did not appear to give the same reliability when used under ANP conditions. Contemporary LC columns and packings are designed for today's instrumentation, not the legacy instrumentation used in the research presented here. Thus, those may be atypical of those found using the latest instrumentation. Also, evaluation of the ANP mode was limited by lack of mass spectrometric detection. However, there are indications that ANP LC would be useful for simple LC analyses for verifying the compositions of tobacco products and e-liquids. With ANP, it may be possible to analyze for new compounds without a new column.