Non-targeted Analysis for Differential Screening of Tobacco Samples

John H. Miller IV, Yezdi B. Pithawalla, Timothy L. Danielson, Dongmei Xu

Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219 USA

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Abstract

Non-targeted analysis of samples has broad applications across the food industry and in the evaluation of natural products. For example, this approach has been used by the food industry to evaluate purity and quality of fruit juices, olive oil and tea. Our lab explored applying a non-targeted analysis using LC/MS-TOF to differentiate between types of tobacco and tobacco leaf blends. This work can provide valuable information to improve our understanding of differences in types of tobacco, curing processes, tobacco origin and the impact of leaf blending. In addition, it may help us identify compounds that contribute to a tobacco's key characteristics. The Waters MarkerLynx XS software was able to find hundreds of marker compounds in a variety of tobacco samples. Additional data processing in the software provided principal component analysis for the markers, and grouped the tobacco samples in scores plots. Loadings plots provided insights as to which markers are responsible for the unique groupings. MarkerLynx XS software allowed for differentiation of tobacco types and leaf blends. Future work to identify key markers compounds using accurate mass analysis could provide insights into the differences in chemical composition of these tobacco samples.

Introduction

- Targeted analysis involves the quantitation of known components to characterize differences in tobacco samples. This is good for quantitative analysis of key components, but there are several limitations:
  - Quantitates a handful of compounds in each analysis
  - Requires multiple techniques to characterize differences
  - Time consuming to compare multiple samples
- Non-targeted screening of samples allows for comparison between samples based on the response of hundreds of components. The limitations associated with this technique include:
  - Only semi quantitative analysis based on response of analytes
  - Identification of key components may require additional analysis

Experimental Method

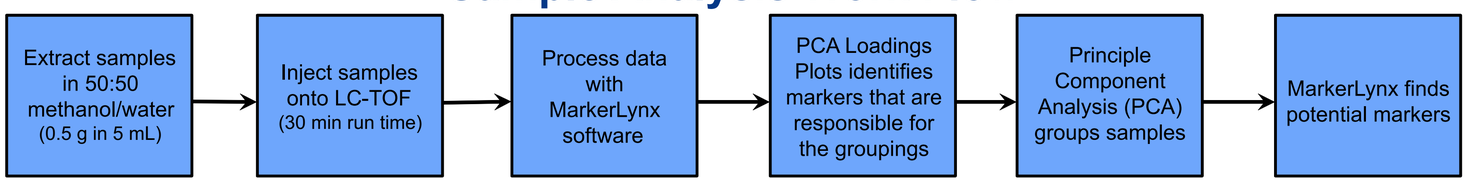
Instrumentation

- Waters Acquity UPLC® with Waters Premier Quadrupole Time of Flight (QTOF) mass spectrometer with positive electrospray ionization in full scan mode
- Chromatographic Separation: BEH C18, 2.1 × 100 mm 1.7 µm column
- Gradient Profile: 5% acetonitrile / 95% ammonium formate to 95% acetonitrile / 5% ammonium formate over 16 minutes

Sample Preparation

- Extract 0.5 g tobacco sample in 5 mL of 50:50 methanol/water
- Samples diluted 1:10 with 2 mM ammonium formate
- All samples analyzed in triplicate

Sample Analysis Work Flow



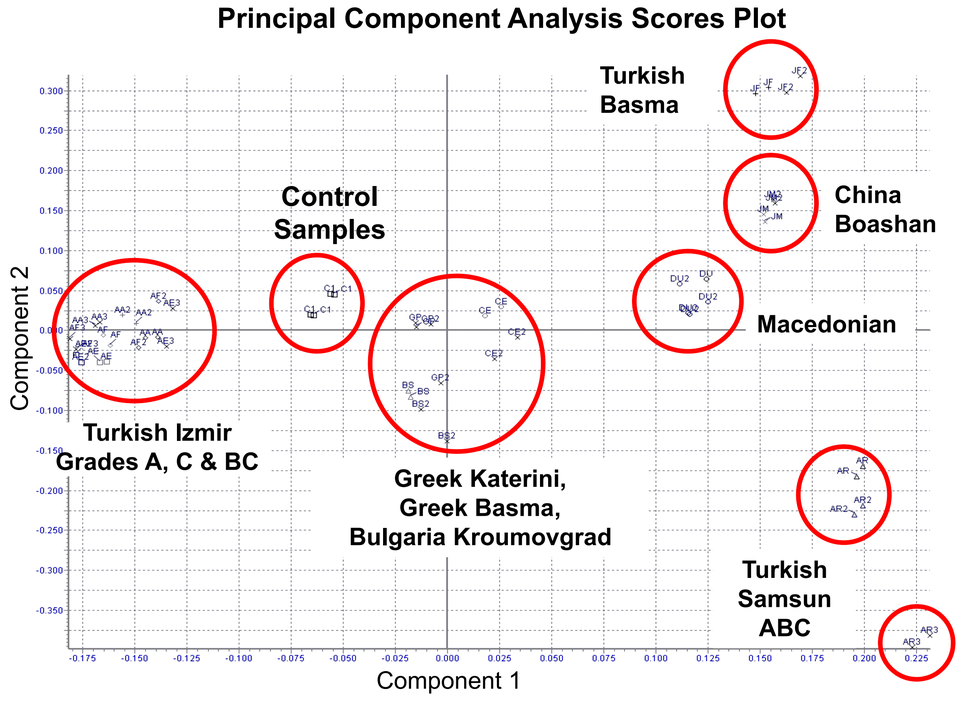
Study 1 – Oriental Tobacco

Background

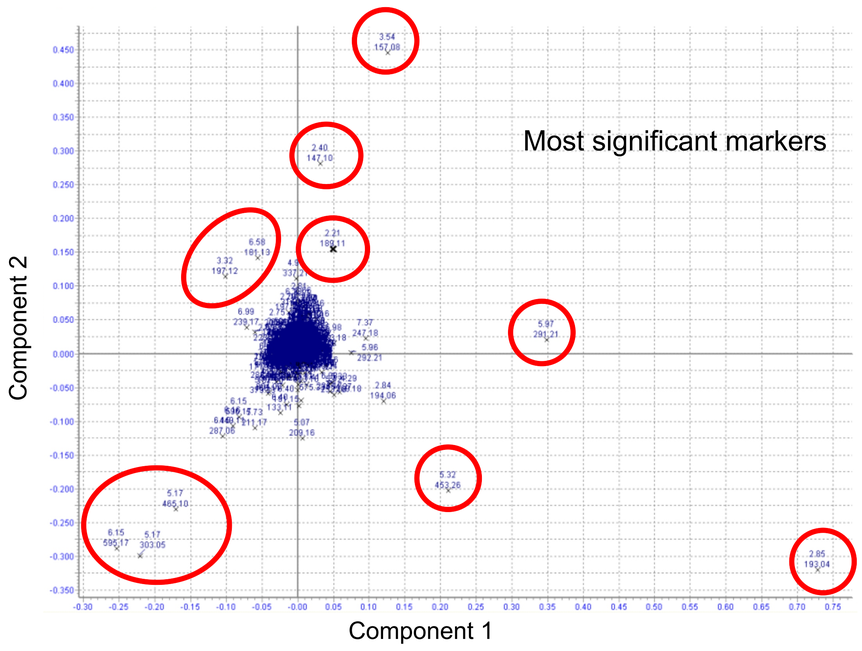
- Oriental, which is also known as Turkish tobacco, is primarily grown in the Aegean region. This is a very dry climate with cooler temperatures than the typical climate where Burley and Bright tobaccos are grown in the United States.
- Oriental tobacco contributes to characteristic aroma and taste in cigarette smoke.
- Oriental tobacco leaves are considerably smaller than Burley or Bright tobacco leaves.
- Project Scope: Evaluate if Oriental tobacco grown in different locations within the Aegean region have unique characteristics that can be identified using MarkerLynx data processing software

Evaluation of Different Varieties Oriental Tobacco Samples

Principal Component Analysis Scores Plot



Principal Component Analysis Loadings Plot determines which markers are significant



Numbers associated with markers represent the chromatographic elution times and corresponding m/z

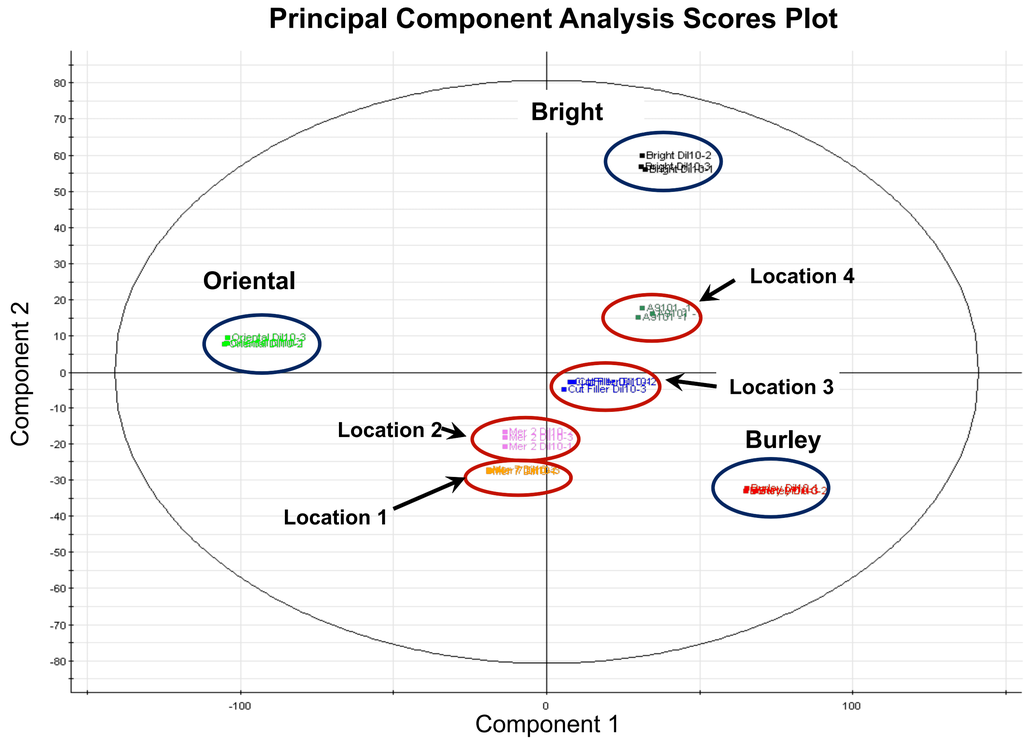
Study 2 – Dust

Background

- Cigarettes are made from a blend of tobacco types including Burley, Bright and Oriental.
- Dust from processing tobacco accumulates in different areas during cigarette manufacturing. Insights into the composition of this dust could help improve manufacturing efficiencies.
- Project Scope: Evaluate if MarkerLynx data processing software could be used to:
  - Differentiate between dust samples collected from 4 different locations in the manufacturing process
  - Identify if the dust samples are higher in composition of a particular tobacco type

Evaluation of Dust Samples Collected During Cigarette Manufacturing

Principal Component Analysis Scores Plot



Summary of Results

Study 1

- MarkerLynx is able to find hundreds of markers in tobacco samples that can be used to discriminate between varieties of Oriental tobacco.
- PCA loadings plots can be used to isolate key markers that impact the groupings.
- Additional work is required to identify these markers using accurate mass analysis.

Study 2

- MarkerLynx is able to differentiate between Burley, Bright, Oriental tobacco and the 4 dust samples that were collected.
- Tobacco dust composition becomes more enriched with Bright tobacco as it moves from Location 1 to 4.

Non-targeted analysis of tobacco samples using MarkerLynx XS Software is a promising tool for differentiation of tobacco types and leaf blends.

References

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2. Silcock P & Uria P. Characterization and Detection of Olive Oil Adulterations Using Chemometrics. Waters Corporation, Application Note, 2008.
3. Twohig M, et al. Adulteration in Fruit Juices: A Solution to a Common Problem. Waters Corporation, Application Note, 2012.

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