Introduction

This study used TIBCO Spotfire® statistical analysis to investigate whether whisky compound profiles are specific to regional origins. Malt whiskies contain a large number of compounds, which vary according to the local ingredients, fermentation, distillation and maturation processes.

Whiskies from different locations in Scotland and Canada were analyzed to detect both involatile organic and inorganic compounds. Data fusion combined the results to produce an extensive component profile for each sample.

Statistical analysis of the profiles clustered samples from similar locations and separated samples from different origins. These results confirm that sample profiles are related to geographic origin and highlight marker compounds that strongly correlate to location.

This approach could be extended to detect adulterated whiskies that do not fit to the component profiles of known single malt and blended whiskies.
Experimental

Whiskies from different geographical origins were analyzed by both accurate mass electrospray LC/MS and by ICP-MS, to create a detailed profile of involatile organic and inorganic components. Commercial Scotch and Canadian whiskies were purchased in plastic miniature bottles. The whiskies used were two Canadian blends, three Scotch blends and one single malt Scotch. Samples of 10 mL were evaporated to dryness at room temperature to remove ethanol and volatile components. Each dried sample was dissolved in water to 10x the original concentration and analyzed by LC/MS to detect the low volatility compounds. Triplicate runs were made on an accurate mass TOF instrument (PerkinElmer AxION® 2 TOF and Flexar™ FX-15 UHPLC system) in both positive and negative modes.

LC conditions:
- Pump: PerkinElmer Flexar FX-15 pump
- Flow: 0.4 mL/min
- Mobile phase A: Water + 0.1% formic acid + 5 mM ammonium acetate
- Mobile phase B: Acetonitrile + 0.1% formic acid
- Gradient conditions: 100% A to 70% B in 10 mins
- Injection volume: 5 µL of concentrated extract
- Column: PKI Brownlee 2 mm x 10 cm C18 UHPLC column

MS conditions:
- Mass spectrometer: PerkinElmer AxION 2 TOF MS
- Ionization source: PerkinElmer Ultraspray™ 2 (Dual ESI source)
- Ionization mode: Positive or negative
- Spectral acquisition rate: 3 spectra/sec

In positive mode, most compounds are detected as [M+NH₄]⁺ or [M+Na]⁺ adduct ions, and in negative mode as [M-H]- ions. Over 100 compounds were detected in total, although a few compounds were detected in both positive and negative modes. Best-fit elemental formulas for many compounds were obtained using the accurate mass and isotopic pattern information with AxION eCID software. Many known compounds could be assigned from the formulas; some were identified as phenolics and terpenes, originating from the oak barrels used to mature the whiskies and from the barley used in the fermentation mash.

For elemental analysis, each whisky was evaporated to dryness, re-dissolved in an acidic solution and analyzed in duplicate in collision mode using a PerkinElmer NexION® 300D ICP-MS instrument.

ICP-MS instrumental operating conditions:
- Nebulizer: Meinhard glass microconcentric
- Spray Chamber: Glass cyclonic
- Triple Cone Interface: Material Nickel/Aluminum
- Plasma Gas Flow: 16.0 L/min
- Auxiliary Gas Flow: 1.2 L/min
- Nebulizer Gas Flow: 1.00 L/min
- Sample Uptake Rate: 250 µL/min
- RF Power: 1600 W
- Replicates per Sample: 3
- Mode of Operation: Collision using He

Data Processing and Statistics

Groups of LC/MS datasets were processed using custom software to reduce complex three-dimension information to a number of significant features with mass, time and intensity information. These features were correlated across all the datasets, while allowing for minor changes to both masses and retention time between analyses, to produce a list of unique (mass, time) pairs; with averaged values across all samples, shown to three decimal places. The processing output is a table of all the features detected in the datasets as columns, with their respective intensities for each sample in rows.

Element concentrations from ICP-MS analysis for each sample were directly exported to a table format from the instrument software. Each of the three tables for (a) LC/MS features in positive and (b) negative modes and (c) ICP-MS element levels were imported into TIBCO Spotfire® software for statistical analysis to differentiate the samples and to visualize the results. New tables were also created by merging features from the separate tables to provide a fuller statistical analysis.

A standard S Plus Principal Component Analysis algorithm was used for statistical calculations, and the resulting scores and loadings tables were visualized in 2D and 3D graphs, color-coding the samples by blend name or geographical origin.

Results

Negative Mode LC/MS

A scores plot (Figure 2) of the principal components from the PCA statistical analysis of the negative mode results showed clearly resolved sample groups, with the Canadian whiskies separated from the Scotch whiskies. The loadings plot (Figure 2) revealed significant markers with significantly different intensities between the sample groups. The (mass, time) feature label for each marker was used to extract accurate masses at that time point in an individual dataset. These mass values were correlated to known chemical compounds in whisky.
Figure 2. Negative node Scores Plot of PC1 v PC2 shows grouping of the two Canadian (red) and four Scotch (blue) samples. Loadings plot displays the marker compounds that most strongly differentiate whiskies in negative mode. Strong markers are m/z 199.1706 (lauric acid), m/z 297.2435 and m/z 171.1389 (capric acid), m/z 300.9978 (ellagic acid), m/z 195.0489 (gluconic acid) and m/z 341.1065 (maltose).

Figure 3. Scores and Loadings plots for the higher mass (> m/z 450) marker compounds that differentiate whiskies in negative mode. Markers include m/z 517.3151 and 487.1820.

Strong differentiators between the whiskies in negative ion mode include capric and lauric acids. These acids derive from barley lipids and remain in the whisky after alembic distillation from copper pots\(^1\). They are detected at higher levels in Scotch whiskies. Another strong differentiator for Canadian whisky was m/z 194.9976, a peak at the start of the separation, identified as containing sulfur by mass and isotopic pattern, which may relate to the caramel that is legally added to these blends. Ellagic acid at m/z 300.9978 (C\(_{14}\)H\(_{14}\)O\(_8\)) is the end product of the degradation of barley tannins and also present in oak wood, and is detected at high levels in certain Scotch whiskies. A non-retained marker at m/z 341.1065 is a disaccharide such as sucrose or maltose, or a mixture of these, higher in two Scotch blends; this compound is also detected in positive mode.
A separate PCA was calculated using only the higher mass range features to highlight the differences for components in this mass range. The loadings plot (Figure 3) shows a marker at m/z 487.1820, more intense in one Canadian whisky, assigned as ring opened cis-oak lactone galloylglucoside and has been previously reported1. A marker at m/z 517.3151 that differentiates the single malt from blends may be an oleanene triterpene from oak wood1. A marker at m/z 515.2144 for a coumaryl glycate is stronger in Canadian blends.

Another view of the information on each sample is produced with a TIBCO Spotfire® bar chart (Figure 4) which summarizes differences. Here the average levels of several color-coded marker compounds are shown for whiskies grouped by country and then by blend.

**Positive Mode LC/MS**

In the positive mode results, one Canadian whisky is the most separated from all other samples in the Scores plot of PC1 v PC2 (Figure 5). The Loadings plot shows that a strong marker is m/z 420.3322, an ammonium adduct of the formula C_{22}H_{42}O_{6} (an unknown compound), which was highest in some Scotch whiskies, and m/z 203.0520 for a glucose adduct. Other markers were noted: m/z 173.0912 for carvone and m/z 110.0587 for a catechol fragment ion (high in one Canadian whisky), m/z 249.1115 (C_{14}H_{16}O_{4}), m/z 127.0387 for hydroxymethylfurfural, m/z 209.0805 for sinapyl alcohol, m/z 193.0495 for scopoletin and m/z 361.2955 for lariciresinol, all present at higher levels in Scotch whiskies.
ICP-MS Results
Statistical analysis of the elemental concentrations from the replicate ICP-MS analysis for each sample highlights the inorganic markers (Figure 6). Due to the wide dynamic range of the concentrations, the square root of each level was used for PCA calculations. The levels of Na were in the range of 2000-8000 µg/L, while the levels of most transition metals were in the range of 0.1-20 µg/L. The single malt has higher levels of a number of metals, especially Cr at a level of ~10 µg/L, whilst one Scotch blend showed higher levels of Sn. These metals are assumed to result from minor impurities in the copper of the alembic stills used for distillation of each whisky. The levels are typical of those reported in other studies of wines and spirits and are well below recommended levels for drinking water.

Integration of Organic and Inorganic Results
A further refinement in the differentiation of whisky blends results from the analysis of the combined information from positive and negative mode LC/MS and ICP-MS analysis. Data fusion of markers requires scaling of the different classes of features before statistical analysis. Intensities for each of the three separate sets of positive and negative organic and inorganic results were scaled to unity values based on the most intense component in that set. (Due to variations of intensity between individual datasets, an average of the top three intensities in each of the positive and negative mode groups was used). The elemental results were then further scaled by a factor of 5 so that the contribution of the largest elemental variation had an equal weight to the most significant LC/MS variations in the PCA calculations. The fusion of the scaled organic and inorganic markers into one table enabled a complete separation of all of the whisky groups in the PCA statistical analysis (Figure 7).

Combined LC/MS Results
Both the positive and negative mode information produced a partial separation of the Scotch and Canadian groups of whiskies in the PCA scores plot, but some Scotch whiskies have very similar patterns of compounds in both ionization modes. Tables for positive and negative mode details were combined to generate a total table of all detected involatile organic markers from the whisky samples. With this fused data, two Scotch blend samples still grouped together. However, the inorganic markers enabled differentiation of these two blends.
Conclusion
The LC/MS and ICP-MS analyses detect chemicals and elements related to the wood, maturation and distillation methods used in whisky production. Here we used data fusion to combine results from independent analyses on the same samples, prior to PCA calculations. The PCA output provides a more complete differentiation of blends than was produced from any single analysis method. The output reveals characteristic markers that aid determination of the origin of different whiskies.

Similar analysis could be used to assign the geographical origins of unknown whiskies and to highlight adulterated and fraudulent samples.

References
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