SHIMADZU
Excellence in Science

Phenols

Sugar and Organic Acids

Method for Whiskey Analysis – Phenols

A method for quantification of whiskey for the phenols present. Whiskey is typically distilled to approximately 70% alcohol content then diluted to the commercially available 40% we are used to seeing. The taste of the whiskey is primarily associated to the values of amphipathic molecules such as guaiacol and peaty taste form cresols.

Method comprises of mixed standards of multiple compounds at similar concentrations. Also includes standard dilutions and investigation into solvent strength of samples.

Whiskey is naturally high in solvents (alcohol) and thus will require dilution with a weaker solvent to prevent poor peak shape during analysis.

Reagents and Safety

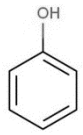
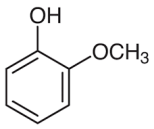
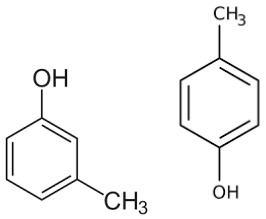
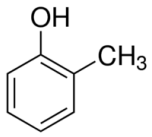
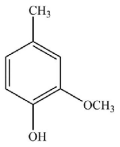
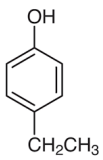
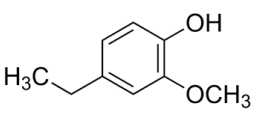
Phenols	-	Harmful if ingested in quantity; corrosive and flammable
Methanol	-	Highly flammable; toxic by ingestion and inhalation
Acetonitrile	-	Highly flammable; toxic by ingestion, inhalation and skin contact; may be mutagen / teratogen

Avoid skin and eye contact with reagents by wearing a lab coat, gloves and safety glasses. Do not expose phenols or methanol to a source of ignition. Avoid inhalation of acetonitrile vapour.

Provided the recommended precautions are adopted, the risk to operators during this procedure is minimal.

Introduction and Structures

A method for quantification of various phenol based compounds within commercially available whiskey's, by high pressure liquid chromatography (HPLC) using a reversed phase column and an external standard as described.

Compound	Structure	Typical Concentration (mg/L)
Phenol		0.017
Guaiacol		0.026
Meta/para-cresol		0.005
Ortho-cresol		0.005
4-methyl guaiacol		0.006
4-ethyl phenol		0.009
4-ethyl guaiacol		0.010
Total Phenols		0.078

Apparatus

- Balance capable of weighing 0.0001 g
- HPLC pump
- Variable wavelength UV detector
- Volumetric flasks
- Glass or electronic pipettes
- Weighing boats

Mobile Phase Preparation

MOBILE PHASE A – Water

- 1 L of HPLC Grade water

MOBILE PHASE B Acetonitrile

- 1L of HPLC or Gradient Grade Acetonitrile

Standard Preparation

Stock Solution (100 mg/L)

- Weigh 10 mg (± 1 mg) of each phenol compound into separate 100 mL volumetrics
- Fill to the mark with Mobile Phase A

Working Standard

- Combine all standards together to prepare a working standard at 1.0 mg/L

  1 mL of each standard in a 100 mL volumetric flask filled to mark with water

Calibration Standards

- Prepare standards from the working standard at the following nominal concentration:

  0.0025, 0.001, 0.005, 0.01, 0.05, 0.1 and 0.5 mg/L in Mobile Phase A
- Run all the above and the working standard for your calibration
- Example of preparation of 0.01 mg/L solution:

  0.1 mL of working standard solution made to mark in a 10 mL volumetric with Mobile Phase A

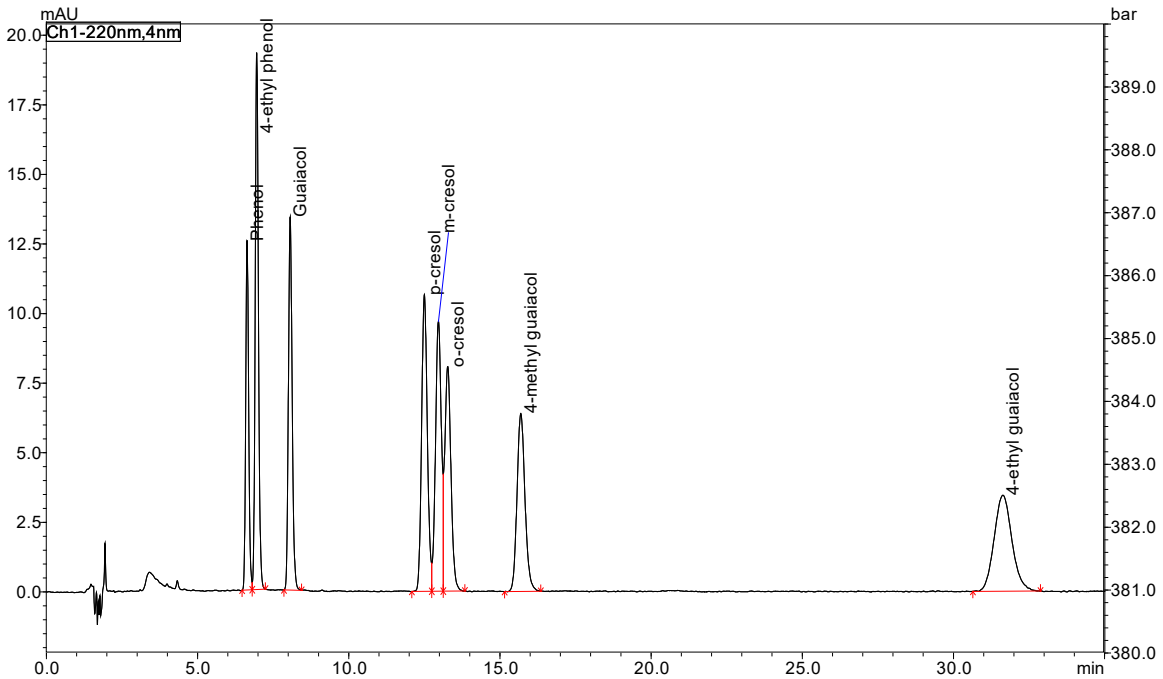
Sample Preparation

- Take a portions of the commercially available whiskey and dilute with water.
- Vary different dilution factors to review changes in the chromatography of the samples

Analytical Conditions

- Column : Phenomenex Kinetex 5.0 μm, F5, 100Å, 250x 4.6 mm
- Temperature : 25°C
- Injected volume : 50 μL
- Mobile phases : A : Water
  : B : Methanol
  : C: Acetonitrile
- Isocratic :63% A : 37% B :
- Flow rate : 1.3 mL/min
- Wavelength : 220 nm (cell at 40 °C)
- Runtime : 35 minutes
- Column Wash : 50:50 Acetonitrile: Water

Typical Chromatography



Information to be recorded

- Weight and Volume of Standard used
- How calibration curve solutions were prepared

- Preparation of each sample solution, varying dilution factors

- Set up of Instrumentation
- Vial positions
- How long column was equilibrated for

- R and R^2 values of calibration curve
- Results for samples, included each phenol and total phenol content
- Investigation results of varying dilution factors

Method for Whiskey Analysis
– Sugar and Organic Acids

A method for quantification of whiskey for the sugars and organic acids present. Whiskey's aldehyde molecules are formed during the fermentation process from interactions of alcohols and organic acids. Acetic acid is the compound providing the characteristic sour flavour of the whiskey. Therefore, monitoring of various sugars and organic acids is essential to ensure batch to batch similarities and production of specific whiskey tastes.

Method comprises of mixed standards of multiple compounds at similar concentrations. Also includes standard dilutions.

Reagents and Safety

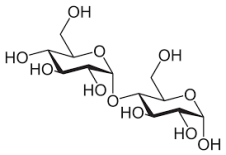
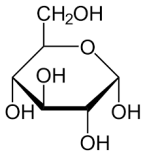
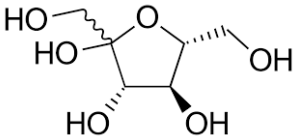
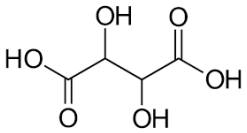
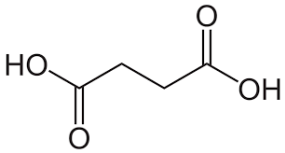
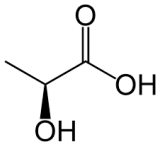
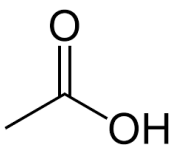
Acids	-	Harmful if ingested in quantity; corrosive
Methanol	-	Highly flammable; toxic by ingestion and inhalation
Acetonitrile	-	Highly flammable; toxic by ingestion, inhalation and skin contact; may be mutagen / teratogen

Avoid skin and eye contact with reagents by wearing a lab coat, gloves and safety glasses. Do not expose Methanol to a source of ignition. Avoid inhalation of acetonitrile vapour.

Provided the recommended precautions are adopted, the risk to operators during this procedure is minimal.

Introduction and Structures

A method for quantification of various sugars and organic acids within commercially available whiskey's, by high pressure liquid chromatography (HPLC) using a reversed phase column and an external standard as described.

Compound	Structure
Maltose	
Glucose	
Fructose	
Tartaric acid	
Succinic Acid	
Lactic acid	
Acetic Acid	

Apparatus

- Balance capable of weighing 0.0001 g
- Volumetric flasks
- Glass or electronic pipettes
- Weighing boats

Mobile Phase Preparation

MOBILE PHASE A – 0.005N Sulfuric acid in Water

- 0.14 mL 95% sulfuric acid to 1L of HPLC grade water

Standard Preparation

Sugar Stock Solution (100 g/L)

- Weigh 10 g (± 1 g) of maltose, glucose and Fructose compounds into separate 100 mL volumetrics
- Fill to the mark with HPLC Grade water

Acids Stock Solution (100 g/L)

- Weigh 1 g (± 0.1 g) of tartaric, succinic, lactic, citric and acetic acids into separate 100 mL volumetrics
- Fill to the mark with HPLC Grade water

Working Standard (50 g/L sugars, and 5 g/L acids)

- Combine all standards together by diluting the two stock solutions in equal portion

Calibration Standards

- Prepare standards from the working standard at the following nominal concentration (concentrations are Sugars/acids):

  1/0.1, 5/0.5, 10/1, 20/2 and 30/3 g/L in HPLC grade water
- Run all the above and the working standard for your calibration
- Example of preparation of 20 g/L solution:

  2 mL working standard in 5mL HPLC Grade water

Sample Preparation

- Take a portion of the commercially available whiskey and dilute with water.
- Vary different dilution factors to review changes in the chromatography of the samples

Analytical Conditions

- Column : Phenomenex Rezex ROA-Organic Acid H+ (8%) 300 x 7.80 mm (8 μm)
- Temperature : 55°C
- Injected volume : 20 μL
- Mobile phases : A : 0.005N Sulfuric Acid (Aqueous) - [See Column Care section]
  : B : HPLC Grade Water
- Isocratic : 100%A
- Flow rate : 0.5 mL/min
- Detector : Refractive Index Detection (RID)
- RID Polarity : +ve polarity
- RID Response : 0.5 sec
- RID Temperature : 40 °C
- Runtime : 30 minutes
- Column Wash : 100% Water [See Column Care section]

Column Care

Resex columns are very sensitive to changes in pressure. The following notes are guidance to settling the column before analysis, cleaning the column post analysis and care of the column.

STARTUP

- Start with a low flow ~0.1 mL/min without the column oven on
- Allow the column to equilibrate at 0.1 mL/min (ambient temperature) for ~20 minutes.
- Stop flow and wipe end outlet of column to remove any particles.
- Restart column flow at 0.1 mL/min for 5 minutes and increase flow by 0.1 mL/min every 5 minutes until required flow of 0.5 mL/min (25 minutes in total).

- After 5 minutes of flow at 0.5 mL/min turn the oven on at 55 °C and allow to warm.
- Whilst oven is warming purge the RID for a minimum of 15 minutes.
- Close the purge valve on the RID
- When both the pressure and baseline are constant analysis can begin.

Step	Duration (mins)	Flow (mL/min)	Column Oven
1	20	0.1	ambient
2	n/a	Stop flow and wipe end outlet of column to remove any particles	
3	5	0.1	Ambient
4	5	0.2	
5	5	0.3	
6	5	0.4	
7	5	0.5	
8	n/a	0.5	Open RID Purge
9	5	0.5	Set at 55 °C
10	After 15 minutes open	0.5	Close RID Purge
11	n/a	0.5	55

SHUTDOWN

Cleaning the column should be done using purified water at 0.4 mL/min at 85 °C for 12 hours

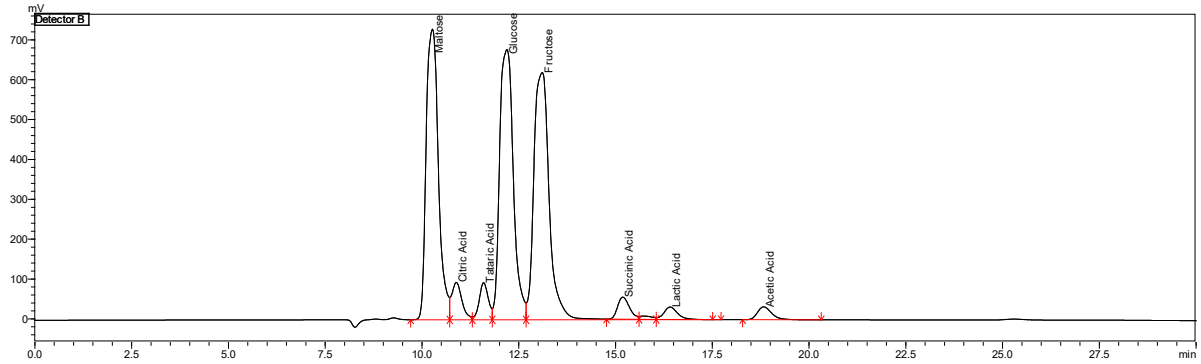
After the 12 hours the oven should be stopped with the flow still at 0.4 mL/min, once cooled the flow can be reduced as per startup to 0.1 mL/min and stopped.

REGENERATION

If the column requires regeneration (peaks retention moves or shape changes) then this can be done using 0.0025 M sulfuric acid in purified water (prepare by addition of 1.4 mL of 95% sulfuric acid in 1 L purfied water).

Using a flow of 0.2 mL/min at 85 °C for up to 16 hours (12 recommended) should regenerate the column.

Typical Chromatography



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