SHIMADZU
Excellence in Science

Soft Drinks - Dyes | Soft Drinks - Sugars | Energy Drinks

Method for Soft Drinks Analysis – Dyes

E-numbers within food have had a lot of investigations over the years. In 2007 scientists showed a link between certain e-number colourants and hyperactivity, especially within children. It is for these reasons that several e-numbers are suggested to be avoided by anyone suffering from hyperactivity or similar medical issues.

These compounds are E102 (tartrazine), E104 (quinoline yellow), E110 (sunset yellow FCF), E122 (Carmoisine), E124 (Ponceau 4R) and E129 (Allura Red). E133 (Brilliant Blue FCF) is generally considered nontoxic and safe, but has previously been banned within some European countries, but has since been allowed within food.

Over the last 20-30 years many artificial e-number colourants have been removed from food, specifically food aimed at children. It is still found within many commercially available soft drinks that are seeing an increase in consumption within children in the UK.

This analytical method will look at varying food dyes and similar dyes within commercially available soft drinks in the UK.

Reagents and Safety

Dyes	-	Harmful if ingested in quantity; can cause skin pigmentation, and have known toxicity
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 solvents to a source of ignition. Avoid inhalation of acetonitrile vapour.

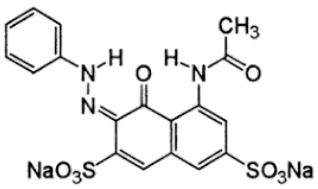
Provided the recommended precautions are adopted, the risk to operators during this procedure is minimal.

Introduction and Structures

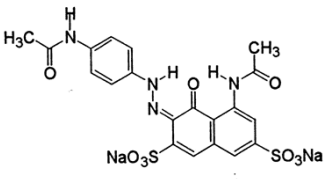
A method for quantification of various phenol based compounds within commercially available whiskey's, by high pressure liquid chromatography (HPLC) using a reversed phase column and an external standard as described.

Compound	E-number	Colour	Retention Time (mins)
Acid Red 1	E128	Red	6.14
Acid Violet 7	n/a	Purple	5.42
Allura Red AC	E129	Red	5.77
Amaranth	E123	Red	4.11
Brilliant Blue FCF	E133	Blue	7.38
Congo Red	n/a	Red	8.32
Fast Green FCF	E143	Green	7.20
Patent Blue V	E131	Blue	8.95
Ponceau 4R	E124	Red	5.04
Sunset Yellow FCF	E110	Yellow	5.30
Tartrazine	E102	Orange	3.69

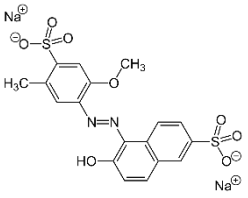
Structures:



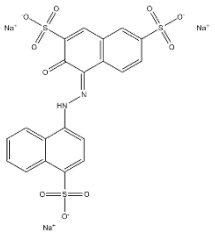
Acid Red 1



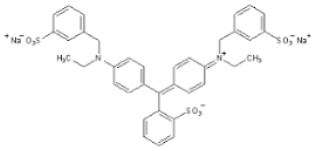
Acid Violet 7



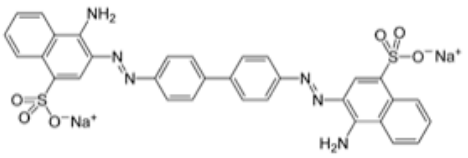
Allura Red AC



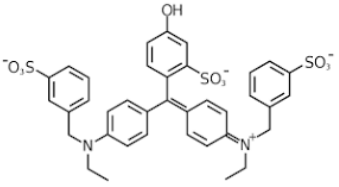
Amaranth



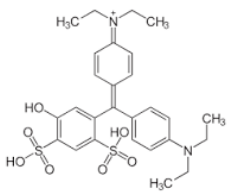
Brilliant Blue FCF



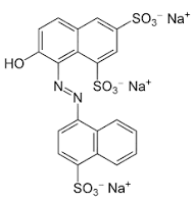
Congo Red



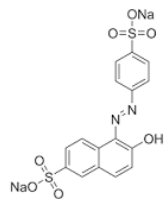
Fast Green FCF



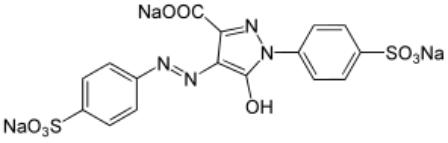
Patent Blue V



Ponceau 4R



Sunset Yellow FCF



Tartrazine

Apparatus

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

Mobile Phase Preparation

MOBILE PHASE A – 20mM Ammonium Acetate in Water

- 1.54g Ammonium Acetate in 1L HPLC Grade Water

MOBILE PHASE B Acetonitrile

Standard Preparation

Stock Solution (2000 mg/L)

- Weigh 20 mg (± 1 mg) of each dye compound into separate 10 mL volumetrics
- Fill to the mark with HPLC Grade Water

Working Standard

- Combine all standards together to create a mixed working standard at 50.0 mg/L

  2.5 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:

  0.5, 1.0, 2.5, 5.0, 10.0 and 25 mg/L in HPLC Grade water
- Run all the above and the working standard for your calibration

Sample Preparation

- Take a portion of the commercially available soft drink in a vessel twice the size of your sampling
- Degas the solution in a sonic bath.
- Run with no further sample preparation

Samples that can be used are as follows:

Brand	Flavour	Dyes included
Oshee	Multi Fruit	E133
Monster	Hamilton	E102 and E129
Monster	Ultra Watermelon	E129
Monster	Ultra Gold Pineapple	E102 and E129
Monster	Pipeline Punch	E129
Monster	Monarch	E129
Monster	Ultra Peachy Keen	E102 and E110
Lucozade	Blue Burst	E133
Lucozade	Original	E110 and E124
Red Bull	JuneBerry	E133
IrnBru	Original	E110 and E124

Analytical Conditions

- Column : Shimadzu Velox 150 x 4.6 mm 5μm
- Temperature : 40°C
- Injected volume : 5 μL
- Mobile phases : A : 20mM Ammonium acetate in Water

  : B : Acetonitrile

  : C: Water
- Gradient :

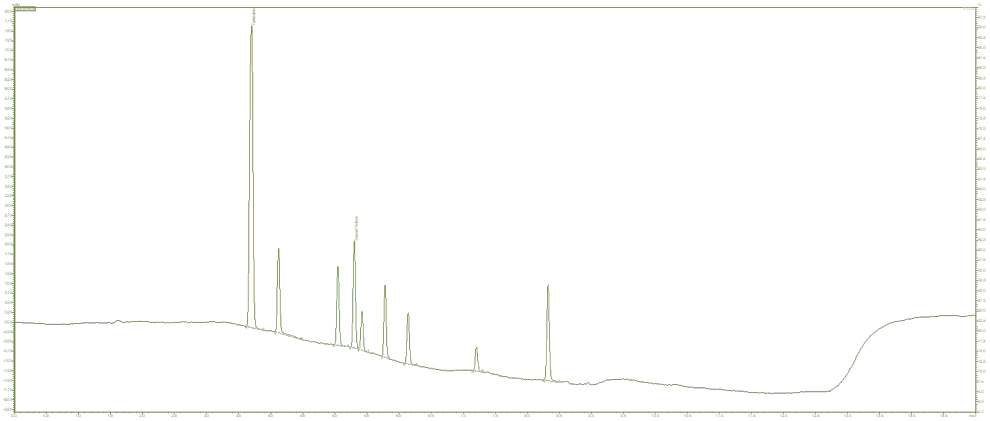
Time	%A	%B
0	98	2
1	98	2
10	50	50
11	50	50
11.1	98	2
15	98	2

- Flow rate : 1.0 mL/min

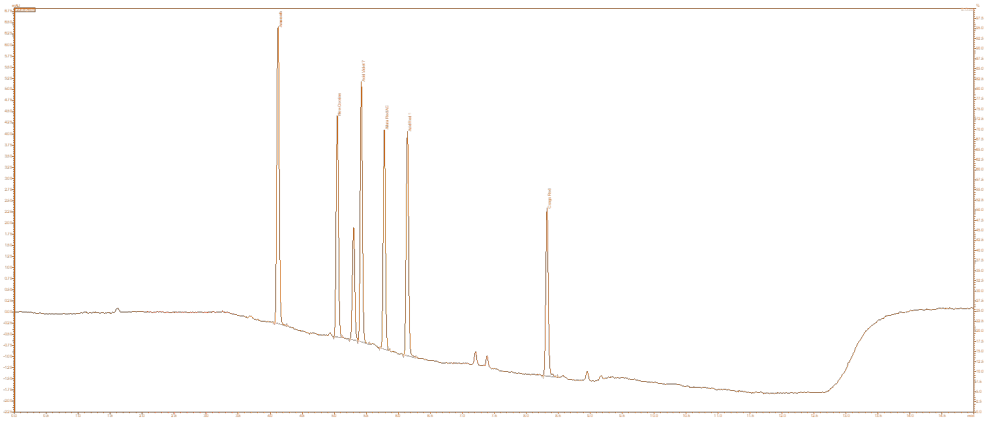
- Wavelength : 450 nm Yellow, 520 nm Red, 620 nm Blue (cell at 40 °C)
- Runtime : 15 minutes
- Column Wash : 50:50 Acetonitrile: Water

Typical Chromatography

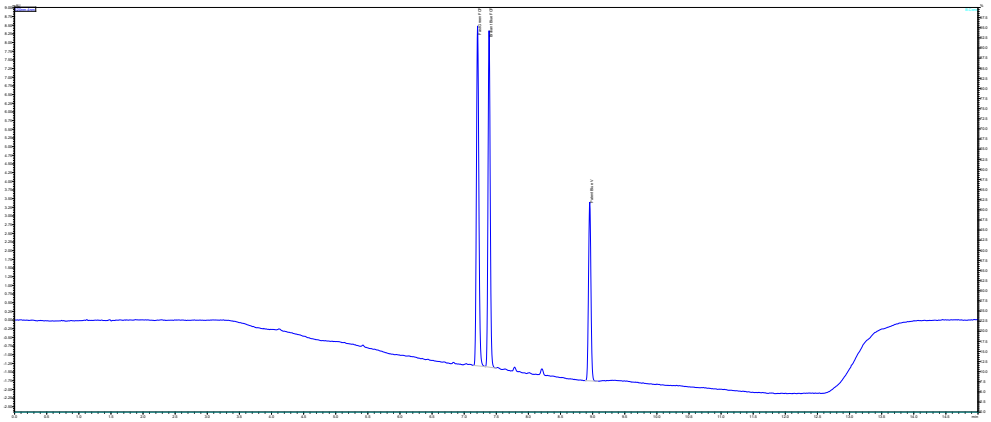
Yellow



Red



Blue



Information to be recorded

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

- Preparation of each sample solution

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

- R and R^2 values of calibration curve
- Results for samples, which dyes and quantity

Method for Soft Drink Analysis – Sugars

Sugar in soft drinks varies from drink to drink, however the levels of which sugars are present also alters. This method looks at the most common four sugars and their individual concentrations within soft drinks.

Reagents and Safety

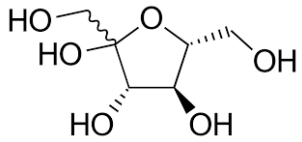
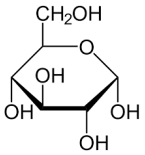
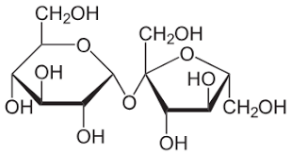
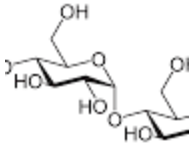
Phenols	-	Harmful if ingested in quantity; corrosive and flammable
Acetonitrile	-	Highly flammable; toxic by ingestion, inhalation and skin contact; may be mutagen / teratogen

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 sugars available soft drinks, by high pressure liquid chromatography (HPLC) using a reversed phase column and an external standard as described.

Compound	Structure
Fructose	
Glucose	
Sucrose	
Maltose	

Apparatus

- Balance capable of weighing 0.0001 g
- 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 g/L)

- Weigh 10 g (± 0.5 g) of each sugar into separate 100 mL volumetrics
- Fill to the mark with HPLC Grade Water

Calibration Standards

- Prepare the highest concentration calibration standard by combining 1 mL of each of the 5 sugar standards (10 g/L) in HPLC Grade water.
- Prepare the rest of the calibration standards from the highest concentration 10 g/L standard in the following concentrations 0.5, 1.0, 2.5 and 5.0 in HPLC grade water
- Example of preparation of 2.5 g/L solution:
  500 µL of the 5.0 g/L standard plus 500 µL of water
- Run all 5 standards as part of the calibration

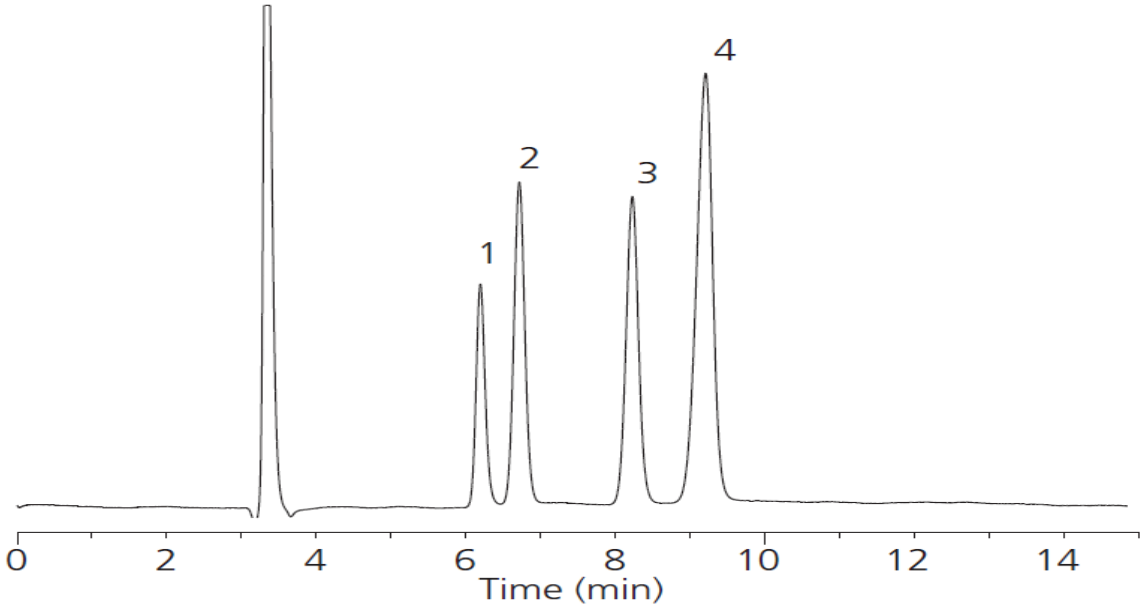
Sample Preparation

- Take a portion of the commercially available soft drink in a vessel twice the size of your sampling
- Degas the solution in a sonic bath.
- Run with no further sample preparation

Analytical Conditions

- Column : Shimadzu Shim-pack GIST NH_2, 250 x 4.6mm, 5 μm
- Temperature : 40°C
- Injected volume : 10 μL
- Mobile phases : A : Water
  : B : Acetonitrile
- Isocratic : 25% A : 75% B
- Flow rate : 1.0 mL/min
- Refractive Index : Purged cell at 40 °C
- Runtime : 15 minutes
- Column Wash : 50:50 Acetonitrile: Water

Typical Chromatography



1 – Fructose, 2 – Glucose, 3 – Sucrose, 4 - Maltose

Information to be recorded

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

- Preparation of each sample solution

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

- R and R^2 values of calibration curve
- Results for samples, included each cannabinoid present and its quantity.
- Are your results the same as the company have stated are present.

Method for Energy Drinks Analysis
- Caffeine, Citric Acid and B Vitamins

Most energy drinks contain the same specific ingredients in varying concentrations. These compounds are citric acid, caffeine and B vitamins B3, B5, B6 and B12. The following method looks at the analytical method for determining the concentration of these compounds in varying energy drinks commercially available.

Reagents and Safety

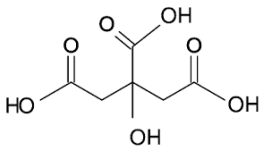
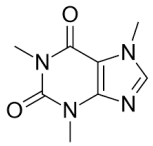
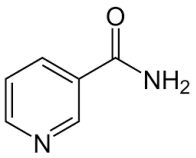
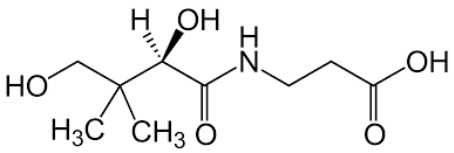
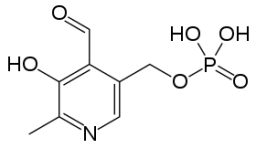
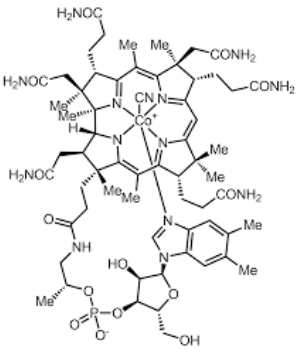
Methanol	-	Highly flammable; toxic by ingestion and inhalation
Acetonitrile	-	Highly flammable; toxic by ingestion, inhalation and skin contact; may be mutagen / teratogen

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
Citric Acid	
Caffeine	
Vitamin B3	
Vitamin B5	
Vitamin B6	
Vitamin B12	

Apparatus

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

Mobile Phase Preparation

MOBILE PHASE A – Phosphate Buffer in Water

- 7.8 g sodium dihydrogen phosphate dissolved in 800 mL of HPLC Grade water
- pH adjust to pH 2.8 with phosphoric acid
- Fill to 1L with HPLC Grade water

MOBILE PHASE B Acetonitrile

- 1L of HPLC or Gradient Grade Acetonitrile

Standard Preparation

Stock Solutions (varying)

- Prepare the following standards by weighing the appropriate amount and dissolving in 10 mL of water. Sonicate if necessary.

Compound	Weight (mg)	Concentration (mg/mL)
Vitamin B3	20	2
Vitamin B5	40	4
Vitamin B6	5	0.5
Vitamin B12	0.45	0.045
Caffeine	100	10
Citric Acid	1200	120

Calibration Standards

- Prepare mixed calibration standard as per the instructions below:

Standard ID	Volume of each stock solution (mL)	Volumetric (filled to lin with water) [mL]
Standard 1	3	20
Standard 2	2	20
Standard 3	1	20
Standard 4	0.5	20
Standard 5	0.25	20
Standard 6	0.1	20

Alternative methods to produce the above can be used if required.

Sample Preparation

- Take a portion of the commercially available soft drink in a vessel twice the size of your sampling
- Degas the solution in a sonic bath.
- Run with no further sample preparation

Analytical Conditions

- Column : Shimadzu Shimpak GIST C18-AQ 150 x 4.6 mm 5μm
- Temperature : 40°C
- Injected volume : 10 μL
- Mobile phases : A : Phosphate Buffer (Water)

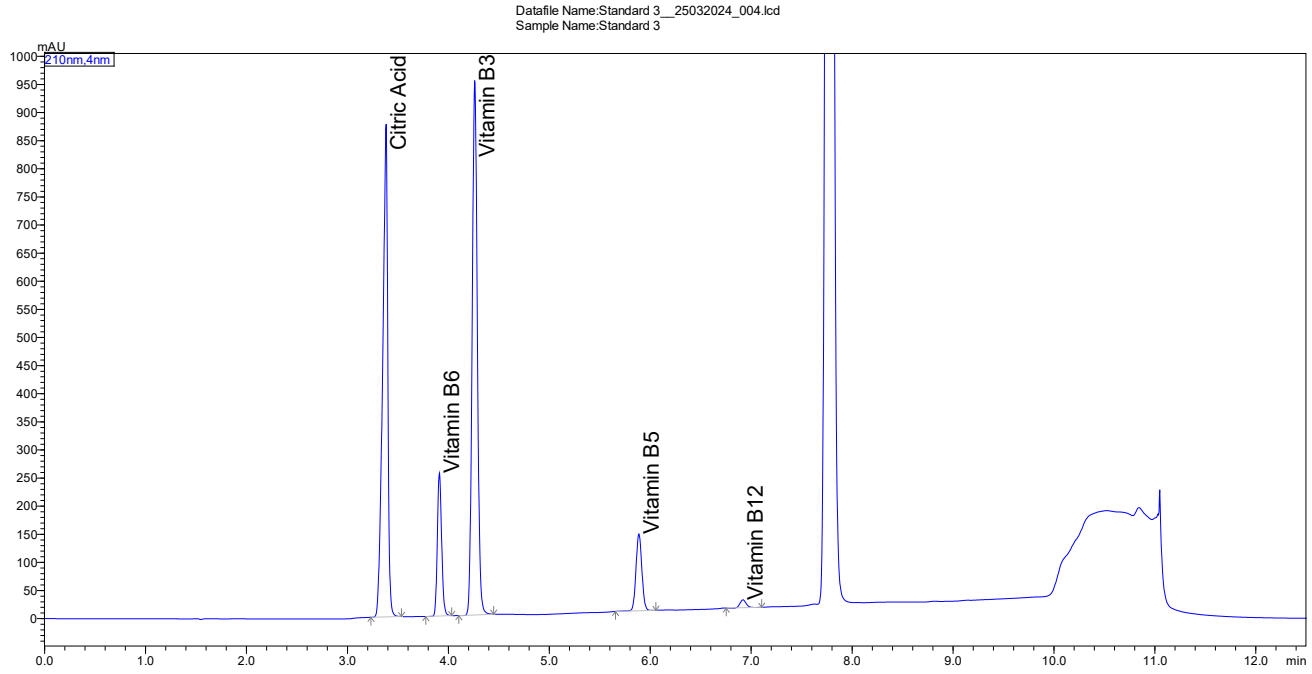
  : B : Acetonitrile

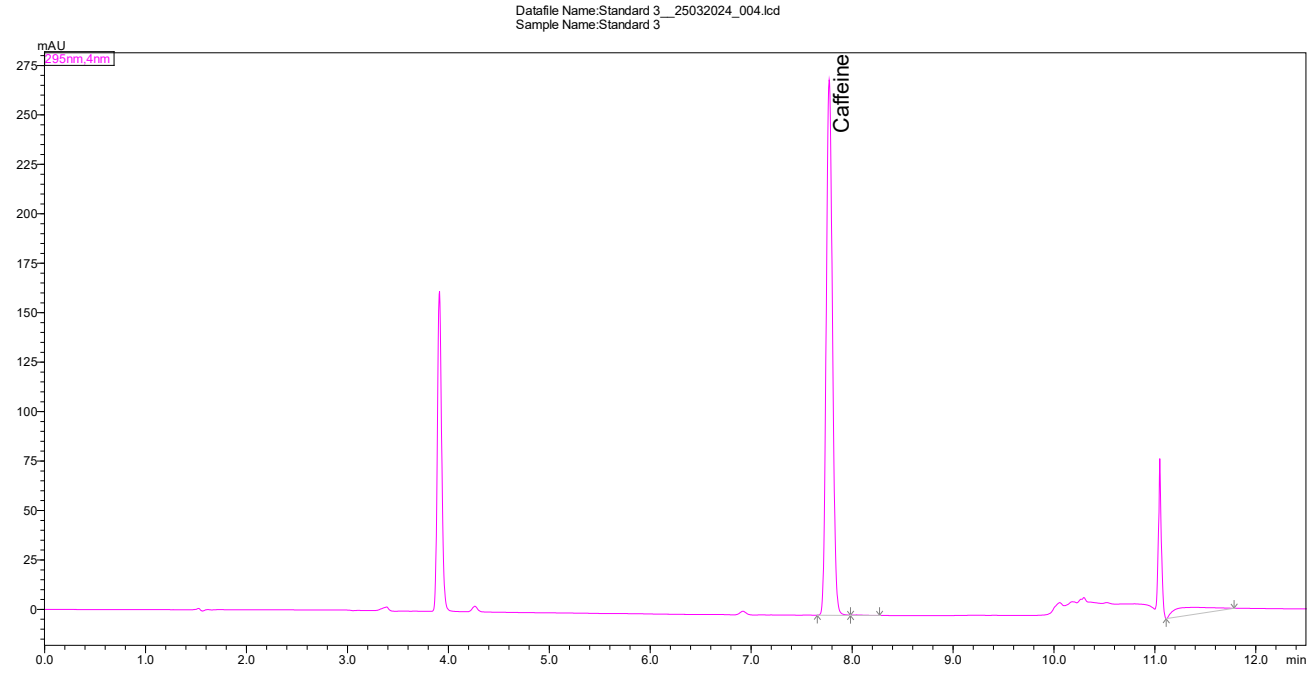
  : C: Water
- Gradient :

Time	%A	%B
0	100	0
8	40	60
8.1	1	99
9	1	99
9.1	100	0
12.5	100	0

- Flow rate : 1.0 mL/min
- Wavelength : 210 nm and 295 nm for caffeine (cell at 40 °C)
- Runtime : 12.5 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

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

- R and R^2 values of calibration curve
- Results for samples, included each compound present and its quantity.
- What energy drink had the highest caffeine content?