

Simultaneous determination of caffeine and benzoic acid from chocolates by chromatographic techniques

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Abstract

A simple, sensitive and precise high performance liquid chromatography method for the analysis of Caffeine and Benzoic acid has been developed, validated and used for the determination of compounds in food products. The compounds were well separated on a C18 column, Agilent, Eclipse XDB C18, (4.6cm x 100mm, i.d., 3.5 μ m) utilizing a mobile phase consisting of phosphate buffer : Methanol (70:30, v/v, pH 3.0) at a flow rate of 1.0ml/min with UV detection at 220 nm. The retention time of Caffeine was found to be 2.5min and retention time of Benzoic acid was found to be 8.4 min. The procedure was validated for linearity (Correlation coefficient = 0.9998). According to the validation results, the proposed method was found to be specific, accurate, precise and rapid. Hence the same can be applied to the quantitative analysis of Aerated drinks and Chocolates containing Caffeine and Benzoic acid.

Keywords: Caffeine, Benzoic acid, High performance liquid chromatography, C18 column, Method validation

1. Introduction

Caffeine is naturally occurring substance found in the leaves, seeds or fruits of over 63 plants species worldwide and is part of a group of compounds known as methylxanthines [1-5]. The most significant sources of caffeine are coffee, tea, Guarana, cola nuts and cocoa [3, 5, 6, 7]. The amount of caffeine found in these products varies, the highest amounts is found in Guarana (4-7%), followed by tea leaves (3.5%), coffee beans (1.1-2.2%), cola nuts (1.5%) and cocoa beans (0.03%) [8, 9]. Pure caffeine occurs as odorless, white, fleecy masses, glistening needles of powder. Its molecular weight is 194.19 g, melting point is 236°C, point at which caffeine sublimates is 178°C at atmospheric pressure, pH is 6.9 (1% solution), specific gravity is 1.2, volatility is 0.5%, vapor pressure is 760mm Hg at 178°C, solubility in water is 2.17%, vapor density 6.7 [5, 8, 10, 11].

Although preservatives have been used for centuries, there has always been some concern

Regarding the safety of consuming food additives used in foods [1]. Benzoic acid (CAS No. 65-85-0; C₇H₆O₂; C₆H₅COOH), can also be called benzene carboxylic acid or phenyl carboxylic acid and usually represented as E210 on the labels of foodstuffs [4]. Benzoic acid is a colourless white

crystalline solid. It melts at 122 °C, boils at 249 °C and starts to sublime at 100 °C, It has a solubility in water (2.9 g/liter at 20 °C), and its solution in water is weakly acidic (dissociation constant at 25 °C = 6.335 $\times 10^{-5}$; pK_a 4.19). It is soluble in ethanol and slightly soluble in benzene and acetone.

2. Material and Methods

- 1) HPLC: An Agilent 1200 series rapid resolution LC consisting of; G1312B Binary pump SL, G1315C UV/VIS diode array detector SL, flow cell as indicated in individual chromatograms, Column: Agilent, Eclipse XDB C18, (4.6cm x 100mm, i.d., 3.5 μ m).
- 2) UV-Visible Spectrophotometer
- 3) Diluent: 8% acetonitrile + 92% Water contain 2% acetic acid, 0.5% ammonium acetate.
 - i) Mobile phase: 70:30 (Buffer pH 3.0: Methanol)
 - ii) Preparation of standard solutions:

For HPLC determination: Standard solution of Caffeine and Benzoic acid was prepared of 500 ppm concentration. Using that standard solution a series of dilutions ranging from 10 ppm to 50 ppm was prepared.
 - iii) Sample prepared for HPLC

Table 1: Preparation of Sample solution for analysis by HPLC

Sample	Wt. Of sample (mg)	First Dilution(ml)	Conc. Of Solution(ppm)	Volume taken for final dilution (ml)	Final dilution (ml)	Final Conc (ppm)
Dairy milk	50	100	500	5	50	50
Shots	50	100	500	5	50	50
Five star	50	100	500	5	50	50
Choclair	50	100	500	5	50	50
Melody	50	100	500	5	50	50

Caffeine Standard Preparation

Accurately weighed 25mg of Caffeine standard and transferred 50ml volumetric flask. This was dissolved in about 20ml of diluent sonicate for 5 minutes and cool at room temperature, then diluted up to the mark with diluent, mixed well.

Benzoic acid Standard Preparation

Accurately weighed 25mg of Caffeine standard and transferred 50ml volumetric flask. This was dissolved in about 20ml of diluent sonicate for 5 minutes and cool at room temperature, then diluted up to the mark with diluent, mixed well.

Preparation of Working Standard Solution

Pipette out 1.0ml of Caffeine Standard Solution and 1.0ml of Benzoic acid Standard Solution to 10.0ml separately

volumetric flask. Made up the volume up to the mark with diluent, shake well.

3. Result and discussion

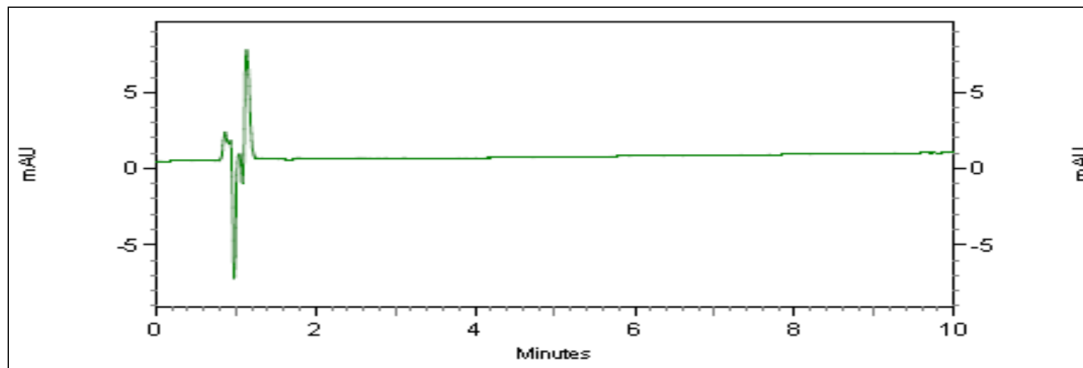


Fig 1: Blank Chromatogram

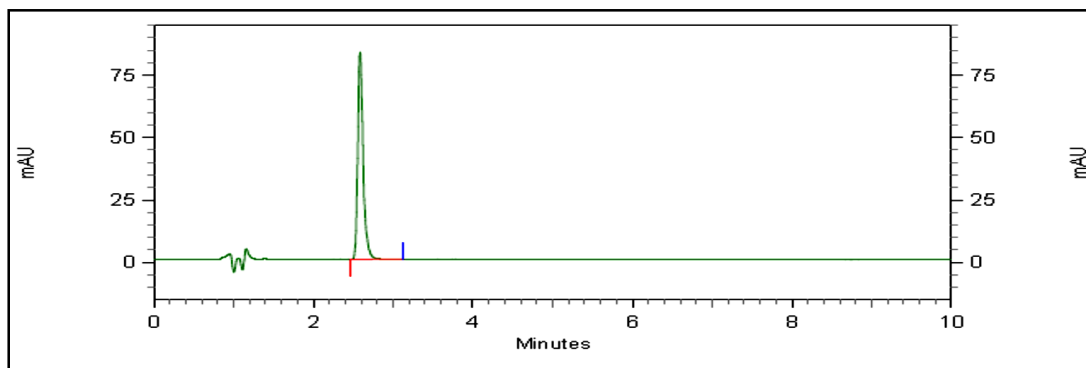


Fig 2: Caffeine Standard

DAD:
Signal
B, 220
nm/Bw:1
6 nm
Results

Pk #	Name	Retention Time	Area	Area Percent	Height	Height Percent	Theoretical plates (USP)	Asymmetry
1		2.587	2806700	100.000	587437	100.000		0.00000
Totals			2806700	100.000	587437	100.000		

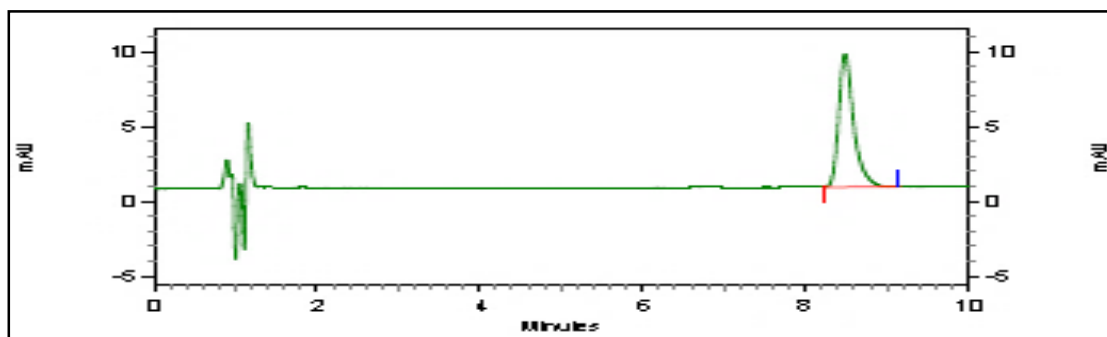


Fig 3: Benzoic acid Standard

DAD:
Signal
B, 220
nm/Bw: 1
6 nm
Results

PK #	Name	Retention Time	Area	Area Percent	Height	Height Percent	Theoretical plates (USP)	Asymmetry
1		8.487	8984355	100.000	699492	100.000		0.00000
Totals			8984355	100.000	699492	100.000		

Table 2: Linearity for Caffeine by HPLC

Sr. No	Conc. In ppm	Observed peak area		Mean area
		1	2	
1	10	1452080	1452162	1452121
2	20	2806700	2815923	2811311
3	30	4341420	4268141	4304780
4	40	5657560	5608833	5633196
5	50	7076721	7078182	7077451

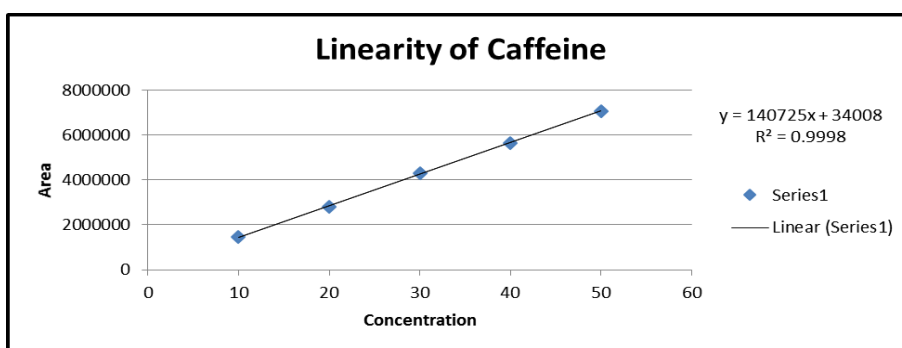


Fig 4

Table 3: Linearity for Benzoic acid by HPLC

Sr. No	Conc. In ppm	Observed peak area		Mean area
		1	2	
1	10	1768412	1769204	1768808
2	20	3513300	3520448	3516874
3	30	5336808	5338721	5337765
4	40	7074443	7073254	7073849
5	50	8984355	8978232	8981294

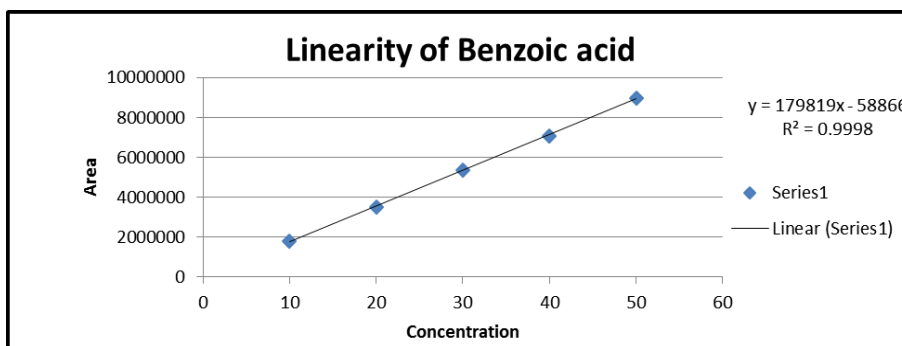


Fig 5

Table 4: Linearity for Caffeine by UV Spectroscopy

Sr. No	Conc. In ppm	Observed Absorbance		Mean Absorbance
		1	2	
1	2	0.1117	0.1124	0.1679
2	4	0.2381	0.2376	0.3569
3	6	0.3838	0.3852	0.5764
4	8	0.5091	0.5157	0.76695
5	10	0.6581	0.6601	0.98815

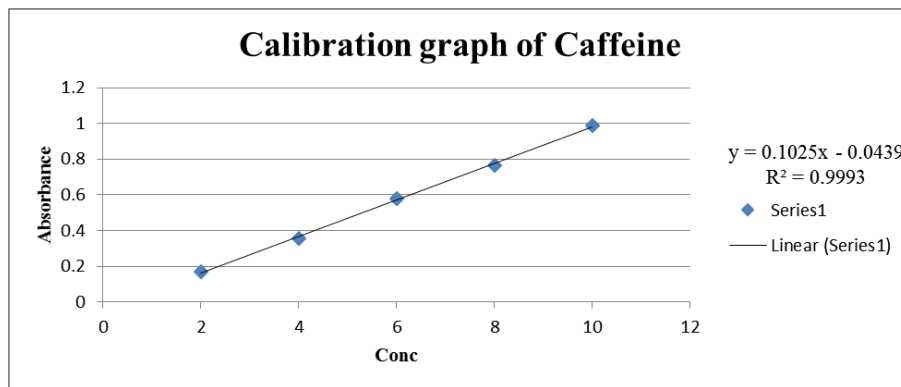


Fig 6

Table 5: Linearity for Benzoic acid by UV Spectroscopy

Sr. No	Conc. In ppm	Observed peak area		Mean area
		1	2	
1	2	0.4497	0.4508	0.6751
2	4	1.0761	1.0698	1.611
3	6	1.8141	1.7182	2.6732
4	8	2.4002	2.4102	3.6053
5	10	3.1135	3.0279	4.6275

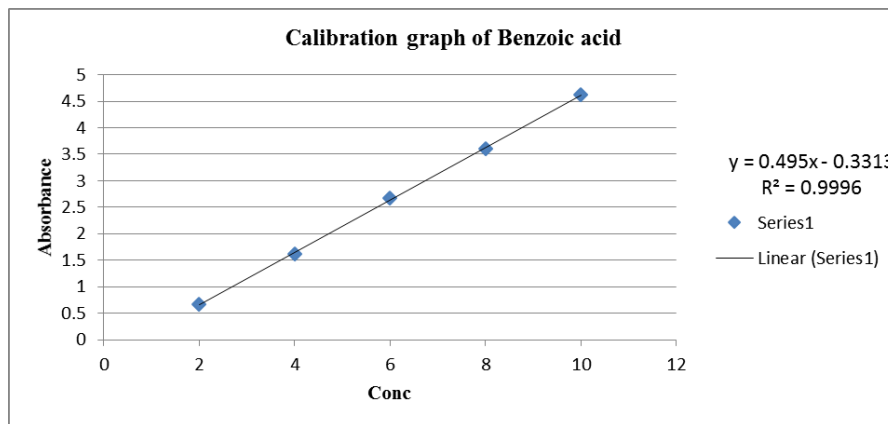


Fig 7

4. Conclusion

Despite the number of methods described by the other researchers for analysis of Caffeine and Benzoic acid, the proposed HPLC method for determination of Caffeine and Benzoic acid in Chocolate samples is simple and rapid than other sophisticated instruments. The procedures are easy to execute and require less sample handling than the methods described in the literature. The following table gives the summary of results.

Calculation

20 ppm Standard Area = 2811311
 Spike (20 ppm Standard + Sample) = 2816169

$$\text{Sample Concentration} = \frac{\text{Standard concentration} \times \text{Sample Area}}{\text{Standard Area}}$$

$$= \frac{20 \times 4858}{2811311}$$

$$= 0.0346 \text{ ppm}$$

Table 6: Caffeine present in Chocolates by HPLC

Sample Name	Diluted Sample % Area	Standard 20ppm % Area	Spike (Sample + 20ppm Standard)	Sample % Area	Caffeine Found (ppm)
Chocclair	4836	2811311	2816169	4858	0.0346
Dairy Milk	70519	2811311	2881873	70562	0.5020
Five star	12412	2811311	2823804	12493	0.0889
Melody	2263	2811311	2813580	2269	0.0161
Shots	5613	2811311	2816943	5632	0.0401

Table 7: Benzoic acid present in Chocolates by HPLC

Sample Name	Diluted Sample % Area	Standard 20ppm % Area	Spike (Sample + 20ppm Standard)	Sample % Area	Caffeine Found (ppm)
Choclair	1915	3513300	3515224	1924	0.0110
Dairy Milk	794	3513300	3514094	794	0.0045
Five star	4825	3513300	3518152	4852	0.0276
Melody	254	3513300	3513568	268	0.0015
Shots	1631	3513300	3514942	1642	0.0093

Calculation

From Linearity graph we found an equation,

$$Y = \text{Slope} \times X - \text{Intercept}$$

$$x = y + 0.0133 / 0.086$$

$$x = 0.0045 + 0.0133 / 0.086$$

$$x = 0.20697 \text{ ppm}$$

Table 8: Caffeine present in Chocolates by UV Spectrophotometer

Sample Name	Calculated absorbance (y)	Slope	Intercept	Found Conc (x)
Diet Coke	0.0045	0.0133	0.086	0.20697
Diet Pepsi	0.0286	0.0133	0.086	0.48720
Pepsi	0.0425	0.0133	0.086	0.64883
Coca Cola	0.0155	0.0133	0.086	0.33488
Mountain Dew	0.0413	0.0133	0.086	0.63488

Table 9: Benzoic acid present in Chocolates by UV Spectrophotometer

Sample Name	Calculated absorbance (y)	Slope	Intercept	Found Conc (x)
Diet Coke	-0.143	0.0133	0.0913	-1.42059
Diet Pepsi	-0.1292	0.0133	0.0913	-1.26944
Pepsi	-0.0877	0.0133	0.0913	-0.81489
Coca Cola	0.0006	0.0133	0.0913	0.15224
Mountain Dew	-0.0507	0.0133	0.0913	-0.40963

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