

## Study of Preservatives and Stimulants in Commercial Soft Drinks

Abida Sultana, Robiul Islam, Md. Mazharul Islam, Mohammad Shoeb and Nilufar Nahar

Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh

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### Abstract

Commercially available soft drinks *i.e.*, Sprite and 7up (lemon) samples of 10 different batches were studied by UV-Vis spectrophotometry to find out the presence of preservative and stimulant. The wavelength of absorption maxima ( $\lambda_{\max}$ ) was 224 nm for sodium benzoate and 272 nm for caffeine. The soft drink samples were degassed, extracted with HPLC grade water and cleaned up to study sodium benzoate and caffeine content. The average quantity of caffeine was in the range of 22 to 30  $\mu\text{g/ml}$  in Sprite and 20 to 30  $\mu\text{g/ml}$  in 7up (lemon), whereas the average quantity of sodium benzoate was in the range of 181 to 191  $\mu\text{g/ml}$  in Sprite and 140 to 160  $\mu\text{g/ml}$  in 7up (lemon) samples. The correlation coefficients of the calibration curves of sodium benzoate and caffeine were found to be 0.9972 and 0.9862, respectively. Recovery experiment was done by spiking sodium benzoate at 20  $\mu\text{g/ml}$  level with 10 replicate studies. The mean recovery of sodium benzoate was  $89.14 \pm 2.14\%$ . The reproducibility and repeatability of the method was very satisfactory with low value of RSD. The present method can be successfully applied for the study of sodium benzoate and caffeine in commercial soft drinks.

**Key words:** Preservative, stimulant, sodium benzoate, caffeine, UV-Vis spectrophotometer

### Introduction

Commercial soft drinks are a combination of different ingredients, including carbonated water, sweetener, artificial flavor, caffeine (Figure 1), coloring agents and preservatives, in particular sodium benzoate (Figure 2). Due to the pleasant taste and ease of availability the demand of commercial soft drinks are increasing all over the world. To meet this demand, preservatives and additives are used to slow down the changes, which cause foods deterioration due to a large number of physical, chemical, enzymatic or biological reactions, and to stop or delay the growth of microorganisms or any deterioration due to microorganisms (Gould, 2000).

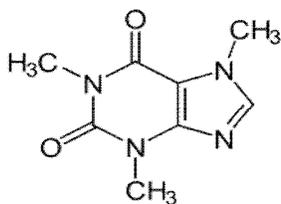


Figure 1. Structure of caffeine.

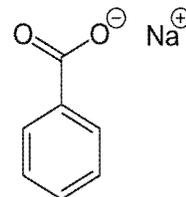


Figure 2. Structure of sodium benzoate.

The use of sodium benzoate as preservative and caffeine as stimulant in soft drink are very common. Sodium benzoate is bacteriostatic and fungistatic under acidic conditions but daily intake can bring adverse effects on skin such as rash, non-immunological contact urticarial, metabolic acidosis, hyperpnoea and asthma (Bronaugh *et al.*, 1982; Coverly *et al.*, 1998; Lahti *et al.*, 1984; Freedman, 1977).

Caffeine is considered as the most frequently used psychoactive substance in the world (Kapil *et al.*, 2011). It is a xanthine alkaloid compound that acts as a stimulant in humans. But excess intake can bring human health problems especially for pregnant women (James *et al.*, 1983; Kirsten *et al.*, 2003). An overdose of caffeine may cause rapid or irregular heartbeat and breathing problem and sleep disorder (American Psychiatric Association, 1994).

Like other countries in the world, consumption of soft drinks and beverages is now a very common habit in Bangladesh. Soft drinks are especially very popular to the children. So, the composition of soft drinks should be regulated by legislations. Determination of food additives and preservatives in soft drinks is mandatory all over the world for ensuring food safety and quality. In continuation of our research on food additives (Abida *et al.*, 2012) in soft drinks and beverages, the present study has been aimed to assess the compliance of the levels of sodium benzoate and caffeine in commercial soft drinks (Sprite and 7up) available from the local market of Dhaka city, Bangladesh.

### Materials and Methods

**Sample collection:** Commercial soft drink samples, Sprite and 7up (lemon) of ten different batches were collected from different areas of Dhaka city during December 2014 and April 2015 and coded as SF<sub>1</sub>-SF<sub>10</sub> and SU<sub>01</sub>-SU<sub>10</sub> for Sprite and 7up, respectively. All of them were in plastic bottle. Samples were stored in a refrigerator.

**Chemicals and reagents:** HPLC grade water (Milli-Q water) which is free from cations, anions and hydrocarbons was used to carry out the study. Standard samples of caffeine and sodium benzoate were obtained by courtesy of Director General of Drug Administration, Government of the People's Republic of Bangladesh.

**Instruments:** Double beam Ultraviolet-visible spectrophotometer (Shimadzu, UV-1800), analytical balance (AL 104, Mettler Toledo), vortex mixture (Kebo LabReax-2000) and centrifuge machine (Cowbell) were used.

**Preparation of standard solutions:** The primary standard (500 µg/ml) solution of sodium benzoate was prepared by dissolving 0.0100 g of sodium benzoate in a 20 ml volumetric flask by using HPLC grade water. Secondary standard solution (200 µg/ml) was prepared by diluting the primary standard and the working standard solutions of sodium benzoate (100, 80, 60, 50, 40, 20, 10, 5, 4, 2, 1, 0.5, 0.4, 0.2, and 0.1 µg/ml) were prepared from secondary standard solution by dilution with HPLC grade water. Similarly, primary

(100 µg/ml), secondary (50 µg/ml) and working standard solutions (40, 20, 10, 8, 6, 5, 4, 3, 2, 1, 0.5, 0.4, 0.2, 0.1 and 0.05 µg/ml) of caffeine were prepared with HPLC grade water. Absorptions of working standard solutions of sodium benzoate and caffeine were measured and calibration curves were drawn by plotting absorption vs concentration graph (Figure 3 and 4) and limit of detections were found out.

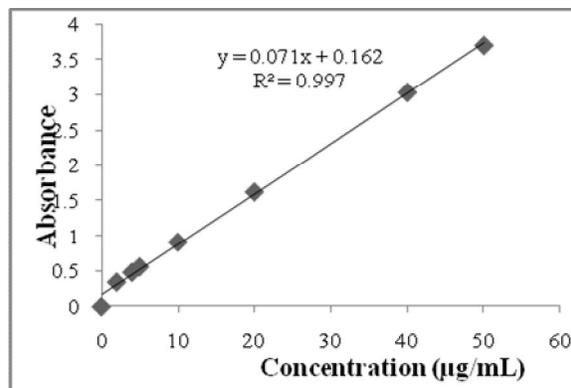


Figure 3. Calibration Curve of sodium benzoate.

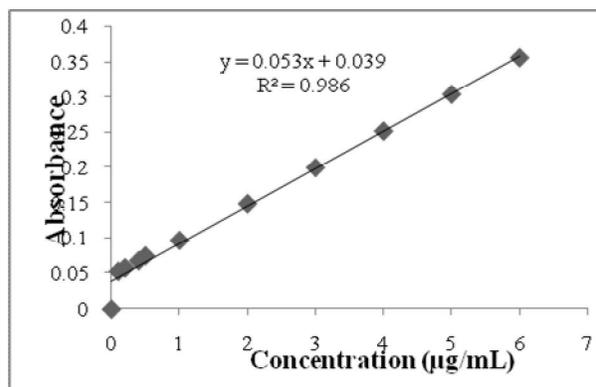


Figure 4. Calibration Curve of caffeine.

**Degassing, extraction and clean up:** 100 ml of each sample of Sprite and 7up was measured by using a cleaned 100 ml volumetric flask. The sample was filtered by a HPLC grade filter paper (0.22 µm) and degassing was done by a degasser machine for about 30 min. The volume of the degassed sample (Table 2) was recorded using burette. 10 ml of the degassed sample was taken in a volumetric flask for dilution. The sample was extracted with HPLC grade water and diluted eight times and homogenized by vortex mixture for 2 min. The solutions were cleaned up by filtering through

HPLC grade syringe filters (0.25  $\mu\text{m}$ ). Absorptions of the cleaned extracts (three replicates for each company of each soft drink) were measured and the concentration of sodium benzoate and caffeine were calculated using calibration curves (Figures 3 and 4). Amount of sodium benzoate and caffeine and their relative standard deviations were calculated and the data are presented in the (Tables 3 and 4).

**Determination of solid mass:** 100 ml of each Sprite and 7up samples with three replications were

evaporated by a rotary vacuum evaporator at about 35-40  $^{\circ}\text{C}$  temperature with the rotation at nearly 200-250 rpm. Then the sample was finally dried to a sticky solid mass by a freeze dryer.

**Recovery experiment:** Recovery experiments were done by spiking sodium benzoate in HPLC grade water at 20  $\mu\text{g/ml}$  concentration level with ten replications. Percent & mean recovery and Relative Standard Deviations were calculated and the results are presented in (Table 1).

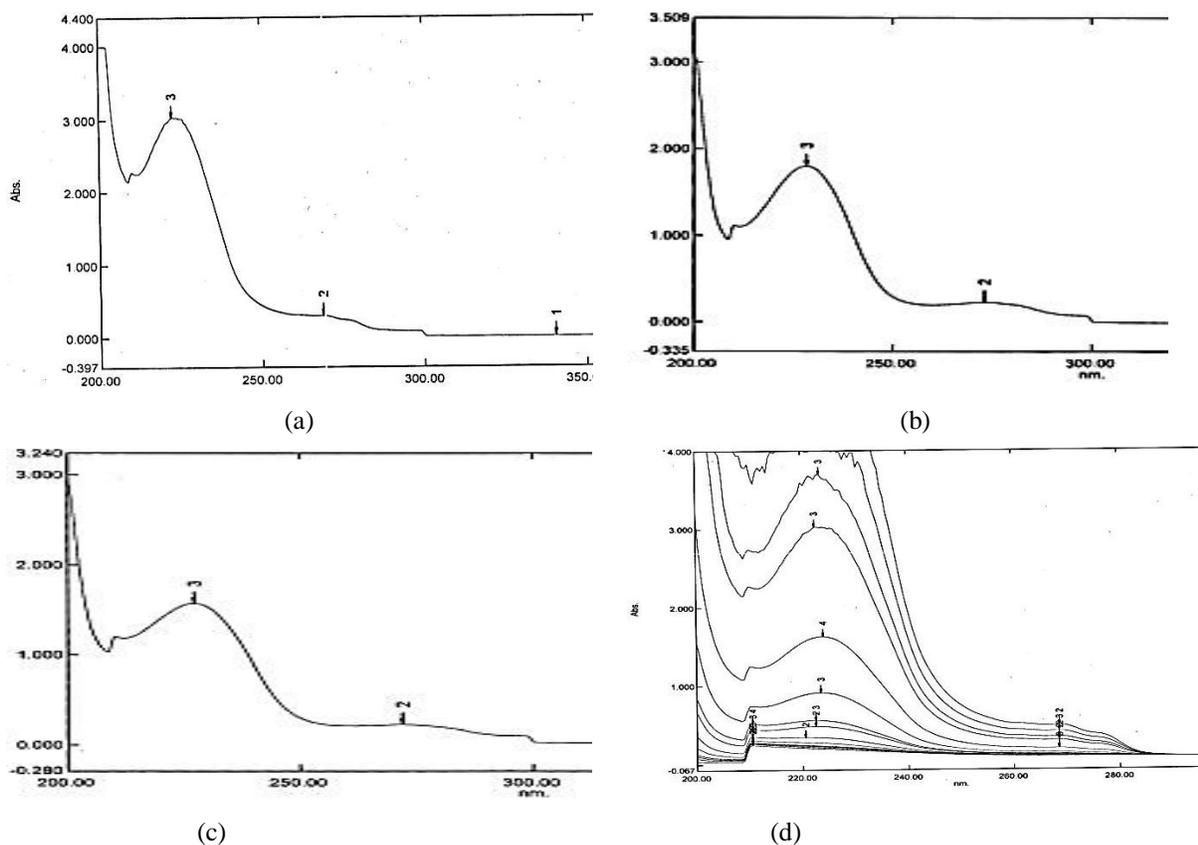


Figure 5. Spectra of standard sodium benzoate (a), cleaned extract of Sprite (b), cleaned extract of 7up (c) and overlapped (d).

## Results and Discussion

The average volume of dissolved  $\text{CO}_2$  gas (Table 2) was 6.04 ml for Sprite samples ( $\text{SF}_1$ - $\text{SF}_{10}$ ) and 7.87 ml for 7up samples ( $\text{SU}_1$ - $\text{SU}_{10}$ ). The dissolved gas was higher in 7up as compared to Sprite samples. The average solid mass content (Bar Graph 1) was  $11.43 \pm 0.02$  g/100 ml of Sprite samples and  $13.09 \pm 0.25$  g/100 ml for 7up samples, respectively. Relatively higher

value of solid mass in 7up indicated higher proportion of additives than Sprite.

The cleaned-up extracts of all the samples of Sprite ( $\text{SF}_1$ - $\text{SF}_{10}$ ) and 7up ( $\text{SU}_1$ - $\text{SU}_{10}$ ) were found to give maximum absorption ( $\lambda_{\text{max}}$ ) at 224 nm for sodium benzoate (Figure 5) and at 272 nm for caffeine (Figure 6). Limit of detection (LOD) of sodium benzoate and caffeine were 1 and 0.1  $\mu\text{g/ml}$  (Figures 5c and 6c)

showed sensitivity of the modified method. The amount of sodium benzoate (Table 3) in Sprite (SF<sub>1</sub>-SF<sub>10</sub>) and 7up (SU<sub>1</sub>-SU<sub>10</sub>) samples were found to be in a range of 181 to 191 µg / ml and 140 to 160 µg/ml. According to FAO/WHO Expert Committee on Food Additives (JECFA), the acceptable daily intake (ADI) of sodium benzoate is 0-5 mg/kg body weight (Vivek *et al.*, 2015). Caffeine content (Table 4) in different batches of Sprite (SF<sub>1</sub>-SF<sub>10</sub>) and 7up (SU<sub>1</sub>-SU<sub>10</sub>) were found to be in a range of 22 to 30 µg / ml and 20 to 30 µg/ml, respectively, whereas the maximum limit of caffeine specified by FDA is 200 µg/ml (Igelige *et al.*, 2014).

Thus, caffeine and sodium benzoate are used in soft drinks of Bangladesh. So, the daily intake of soft drinks mainly for children should be in limited range to avoid adverse effects on health.

The recovery of the used procedure, which reflects the accuracy of the analytical method, was evaluated by analysing preservative free drink sample spiked with 20 µg/ml sodium benzoate (Table 1). The mean recovery of ten measurements obtained by standard addition approach was found to be  $89.14 \pm 2.14$  with relative standard deviation of 2.40%.

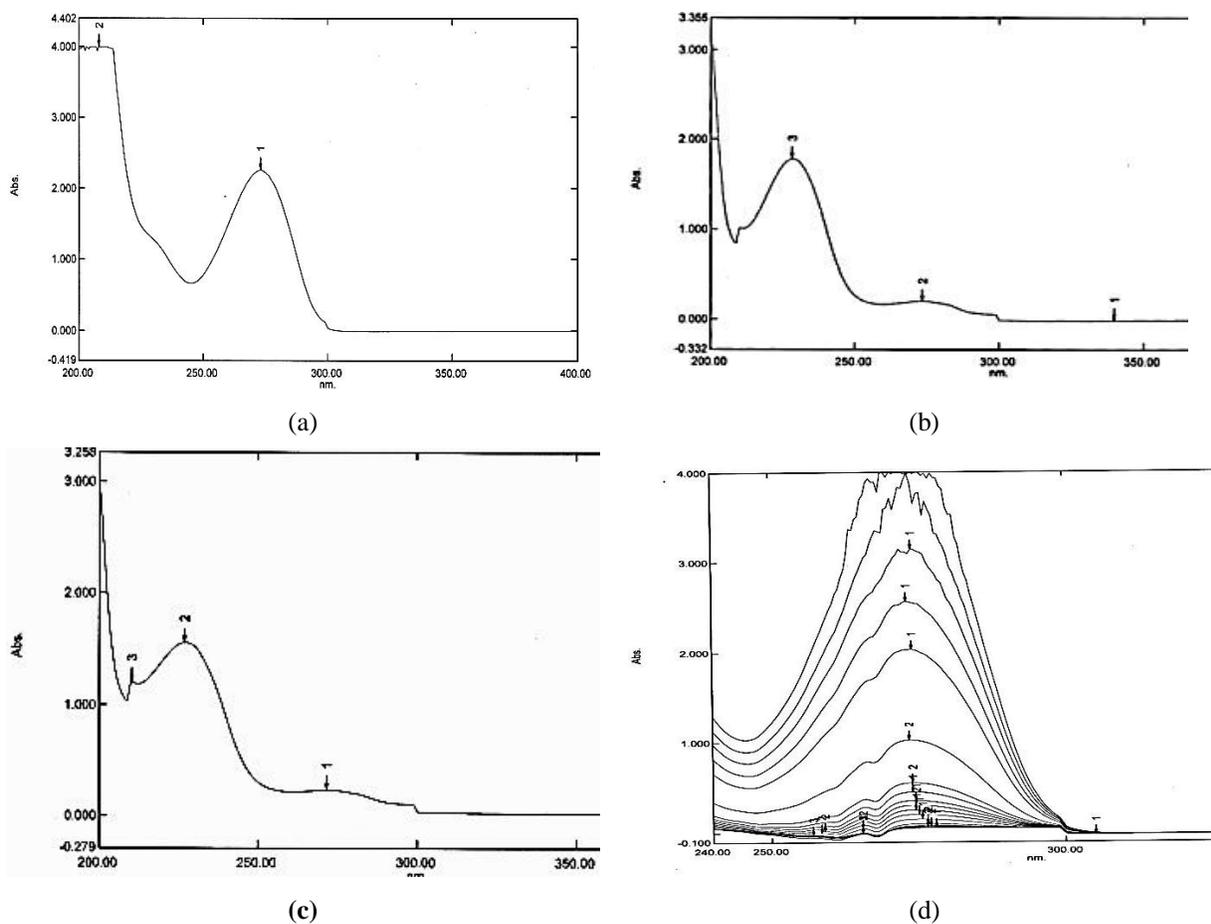


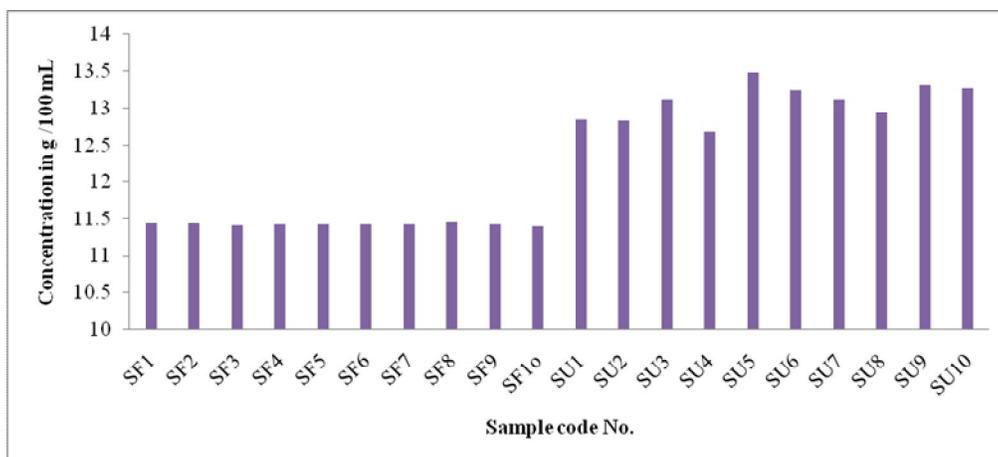
Figure 6. Spectra of caffeine (a), cleaned extract of Sprite (b), cleaned extract of 7UP(c) and overlapped spectra (d).

**Table 1. Results of recovery experiment.**

Spiking concentration of sodium benzoate ( $\mu\text{g/ml}$ )	Absorbance at 224 nm	Concentration of sodium benzoate found ( $\mu\text{g/ml}$ )	Recovery (%)	Mean Recovery (%)	Relative standard deviation (%)
20	1.451	18.04	90.20	$89.14 \pm 2.14$	2.40
	1.389	17.17	85.85		
	1.431	17.77	88.85		
	1.468	18.28	91.40		
	1.441	17.91	89.55		
	1.383	17.09	85.45		
	1.445	17.96	89.80		
	1.418	17.58	87.90		
	1.469	18.29	91.45		
	1.461	18.18	90.90		

**Table 2. Estimation of the volume of dissolved CO<sub>2</sub> gas.**

Sample	Code	Initial volume of the sample (ml)	Final volume of the sample (ml) after degassing	Volume of dissolved CO <sub>2</sub> gas (ml)	Average volume of dissolved CO <sub>2</sub> gas (ml)
Sprite	SF <sub>1</sub>	100	94.10	5.90	6.04
	SF <sub>2</sub>		93.50	6.50	
	SF <sub>3</sub>		94.00	6.00	
	SF <sub>4</sub>		94.15	5.85	
	SF <sub>5</sub>		93.70	6.30	
	SF <sub>6</sub>		93.90	6.10	
	SF <sub>7</sub>		93.98	6.02	
	SF <sub>8</sub>		94.20	5.80	
	SF <sub>9</sub>		94.14	5.86	
	SF <sub>10</sub>		93.90	6.10	
7up	SU <sub>01</sub>	100	90.2	9.8	7.87
	SU <sub>02</sub>		92.5	7.5	
	SU <sub>03</sub>		92.8	7.2	
	SU <sub>04</sub>		94.1	5.9	
	SU <sub>05</sub>		93.6	6.4	
	SU <sub>06</sub>		90.4	9.6	
	SU <sub>07</sub>		92.3	7.7	
	SU <sub>08</sub>		91.8	8.2	
	SU <sub>09</sub>		91.4	8.6	
	SU <sub>10</sub>		92.2	7.8	



Bar Graph 1. Amount of solid mass in soft drinks.

**Table 3. Determination of amount sodium benzoate.**

Sample name	Code	Total amount of sodium benzoate ( $\mu\text{g/ml}$ )	Relative standard deviation ( % )
Sprite	SF <sub>1</sub>	189.55 $\pm$ 2.31	1.22
	SF <sub>2</sub>	190.04 $\pm$ 2.89	1.52
	SF <sub>3</sub>	185.46 $\pm$ 1.59	0.86
	SF <sub>4</sub>	180.92 $\pm$ 3.77	2.08
	SF <sub>5</sub>	181.44 $\pm$ 0.34	0.18
	SF <sub>6</sub>	183.54 $\pm$ 1.94	1.06
	SF <sub>7</sub>	181.06 $\pm$ 1.97	1.09
	SF <sub>8</sub>	187.03 $\pm$ 5.90	3.15
	SF <sub>9</sub>	187.15 $\pm$ 1.84	0.98
	SF <sub>10</sub>	188.01 $\pm$ 0.68	0.36
7up	SU <sub>1</sub>	148.18 $\pm$ 4.094	2.76
	SU <sub>2</sub>	161.02 $\pm$ 1.167	0.72
	SU <sub>3</sub>	151.76 $\pm$ 2.224	1.46
	SU <sub>4</sub>	153.29 $\pm$ 1.398	0.92
	SU <sub>5</sub>	158.64 $\pm$ 2.492	1.57
	SU <sub>6</sub>	160.73 $\pm$ 4.634	2.88
	SU <sub>7</sub>	160.16 $\pm$ 3.535	2.20
	SU <sub>8</sub>	165.99 $\pm$ 4.502	2.71
	SU <sub>9</sub>	160.54 $\pm$ 3.091	1.92
	SU <sub>10</sub>	160.35 $\pm$ 3.895	2.42

**Table 4. Determination of amount of caffeine.**

Sample name	Sample ID	Total amount of caffeine ( $\mu\text{g/ml}$ )	Relative standard deviation ( % )
Sprite	SF <sub>1</sub>	29.65 $\pm$ 1.41	4.76
	SF <sub>2</sub>	27.62 $\pm$ 0.26	0.95
	SF <sub>3</sub>	27.37 $\pm$ 0.95	3.48
	SF <sub>4</sub>	25.86 $\pm$ 1.66	6.45
	SF <sub>5</sub>	22.28 $\pm$ 0.08	0.39
	SF <sub>6</sub>	23.74 $\pm$ 0.23	0.97
	SF <sub>7</sub>	23.74 $\pm$ 1.91	8.07
	SF <sub>8</sub>	25.50 $\pm$ 4.05	15.91
	SF <sub>9</sub>	23.64 $\pm$ 0.23	0.98
	SF <sub>10</sub>	23.44 $\pm$ 0.23	0.97
7up	SU <sub>1</sub>	19.86 $\pm$ 1.06	0.05
	SU <sub>2</sub>	21.61 $\pm$ 0.83	0.04
	SU <sub>3</sub>	21.26 $\pm$ 1.05	0.05
	SU <sub>4</sub>	29.16 $\pm$ 0.22	0.01
	SU <sub>5</sub>	29.12 $\pm$ 0.76	0.03
	SU <sub>6</sub>	29.46 $\pm$ 1.41	0.05
	SU <sub>7</sub>	29.41 $\pm$ 1.05	0.04
	SU <sub>8</sub>	29.96 $\pm$ 0.79	0.03
	SU <sub>9</sub>	29.16 $\pm$ 0.46	0.02
	SU <sub>10</sub>	29.31 $\pm$ 0.86	0.03

### Conclusion

The described UV-Vis spectrophotometric method for the determination of sodium benzoate and caffeine is satisfactory for a wide range of concentration. This method is very much easy to carry out and also cheaper than other analytical methods such as HPLC (Saad *et al.*, 2005; Isabel *et al.*, 2001),

Spectrophotometry (Founi *et al.*, 2002), Capillary Electrophoresis (Kuo *et al.*, 1997), Gas Chromatography-Mass Spectrometry (Hsui *et al.*, 1999) for sodium benzoate and UV spectrophotometric procedures (Ahmad *et al.*, 2005), HPLC (Aresta *et al.*, 2005), Square Wave Voltammetry (Zen *et al.*, 1997) for caffeine. It can be concluded that the method is sufficiently sensitive and reproducible for routine

analysis of sodium benzoate and caffeine in soft drinks within a short analysis time.

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