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Study and Quantification of Preservative (E211) In Corbonated Soft Drink Samples

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Abstract : *Commercial samples of carbonated soft drinks viz, Sprite and Mirinda from three different batches were studied using UV-Vis spectrophotometry to find out the presence of preservatives. The average quantity of sodium benzoate ranged on 168 to 175 µg/ml in Sprite and 396 to 398 µg/ml in Mirinda samples. The correlation coefficients of the calibration curves of sodium benzoate were found to be 0.9985. The mean recovery of sodium benzoate was 87.7 %. The method detection limit and method quantification limit for sodium benzoate was 0.02 and 0.05 mg/L and evaluated to formation of benzene in sample. The present work can be successfully applied for the study of sodium benzoate in commercial carbonated soft drinks.*

Keywords: *Carbonated drinks, Preservative, Sodium benzoate, Spectrophotometric, Vitamin C*

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I. Introduction

Carbonated drinks are beverages, that contain dissolved carbon dioxide [1]. The dissolution of carbon dioxide in a liquid, gives raise to fizz or effervescence. Carbon dioxide is weakly soluble in water, therefore it separates into a gas when the pressure is released. Food preservatives have turned into an essential element nowadays, so that they play an important role during the food transportation [2-3]. Chemical preservatives, such as sodium benzoate (E211) are frequently used in processed foods to prevent the growth of bacteria, yeast or other nasty microorganisms that could spoil your food [4].

Sodium benzoate is used mainly as a preservative in margarine, salad dressings, marinades, cider, soft drinks, pickles, fruit salad, wafers, bakery products, jams, jellies, juices, biscuits, cakes and muffins, tomato paste and soy sauce [5 -8]. There are also reports on the use this substance in wine and beer and olives [9 -11]. Sodium benzoate is bacteriostatic and fungistatic, under acidic conditions, but daily intake can develop adverse effects on skin such as rash, non-immunological contact urticarial, metabolic acidosis, hyperpnoea and asthma [12].

They have been several previous safety assessments undertaken on these substances by several agencies, including Food and Agriculture Organization (FAO),World Health Organization (WHO), Food and Drug Administration (FDA), and Federal Emergency Management Agency (FEMA) [13]. The use of food additives in different countries are limited by specific regulations. According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the safety in use of an additive can be expressed in terms of its acceptable daily intake (ADI), which represents the amount of the substances that can be daily consumed, even for a lifetime, without health hazards [14]. Group ADIs of 0–5 mg/kg of body weight have been established by JECFA for benzoates salts [15].

The E number system for food additives was introduced in the 1960s and the E was intended to reassure consumers that permitted additives were safe. The analytical determination of these preservatives is not only important for quality assurance purposes but alsofor consumer interest and protection. The most common analytical method for determination of benzoate salt is reversed-phase HPLC [16- 18]. The analytical techniques have been much improved by using TLC, gas chromatography, and spectrophotometer have also been reported [19 -21].

Benzoic acid is the most effective antimicrobial agent but benzoates are used more often because of their greater solubility in water. Benzoic acid has low solubility in normal temperatures [22]. Many food and beverage products on the market contain a variety of preservatives which on their own are not necessarily dangerous, but in combination with other ingredients, can form extremely unhealthy, dangerous chemicals. Benzene is obtained through the reaction of sodium benzoate with citric acid and/or ascorbic acid [23-24]. Benzene is recognized by the IARC (International Agency for Research on Cancer) as carcinogenic to humans.

Benzene can form when benzoate is decarboxylated in the presence of ascorbic acid and transition metals such as Cu (II) and Fe (III) and can be accelerated by light and heat [25 - 26]. The study samples are Sprite is a colourless, non-caffeinated soft drink and Mirinda is orange coloured.

The present study has been aimed to assess the compliance of the levels of sodium benzoate and benzene contamination in commercial soft drinks (Sprite and Mirinta) available from local markets.

II. Materials and Methods

2.1 Sample collection:
Commercial soft drink samples, Sprite and Mirinda from three different batches were collected from different areas of Chennai city and coded as SP_1-SP_3 and MIR_1 –MIR_3 respectively. All samples were in plastic bottle and stored under a refrigeration.

2.2 Chemicals and reagents:
HPLC grade water, Sodium benzoate, Citric acid and Ascorbic acid, Methanol (Merck Specialities Pvt Ltd - India)

2.3 Instruments:
Ultraviolet-Visible spectrophotometer (Jasco V-530), Analytical balance (Mettler Toledo), pH meter (Electronic measurement India Pvt Ltd), Water bath and Hot air oven were used.

2.4 Preparation of standard and linearity solutions:
Exactly 100.0 mg of standard sodium benzoate was dissolved in 100.0 mL volumetric flask by using HPLC grade water. Dilution of the stock solutions were made by distilled water to yield 25, 20, 15, 10, 5, 4, 3, 2, 1, and 0.1mg/L of sodium benzoate standard solutions. The absorbance of the standard samples was detected at 225 nm.

2.5 Degassing, extraction clean up and preparation of sample:
The 100 ml of each sample Sprite and Mirinda were degassed using degasser machine for about 20 min. The volume of the degassed sample (Table 1) was recorded using measuring cylinder. 1.0 ml of the degassed sample was taken in a volumetric flask for different dilution. The solutions were cleaned up by filtering through nylon syringe filters (0.2 µm). Absorptions of the solution were measured and the concentrations of sodium benzoate were calculated using calibration curves.

2.6 Physico- chemical properties:
pH was determined by pH meter. Total acidity of sample was measured by titration of sample against 0.1 N sodium hydroxide using phenolphthalein as indicator and expressed as percentage citric acid [27]. Determination of specific gravity and residue on evaporation [27]

2.7 Limit test of Copper and Iron:
Iron and copper tested using Indian Pharmacopoeia (2010).

2.8 Accuracy:
Accuracy was studied by adding known concentration (1.0 ppm) of standard solution to the dilute level of sample preparation such as and comparing the actual and measured concentrations.

2.9 Effect of temperature and transition metal:
Sodium benzoate is decarboxylated to form benzene in the presence of vitamin C (Citric acid or Ascorbic acid) and transition metals such as Cu (II) and Fe (III) and can be accelerated by light and heat. Soft drink samples were incubated at three different temperatures (25 deg C, 45 deg C, and 90 deg C) for 21 days prior to the determination of benzene and that resulted in a significant increase in the levels of benzene in samples subjected to temperatures of 45 °C (ranging from 5.5 ppb to 6.6 ppb) and 90 °C (ranging from 25 ppb to 55.1 ppb) compared to the stored samples 25 °C (ranging from 0.7 ppb to 1.5 ppb). [28].

III. Results and Discussion

3.1 Estimation of the dissolved CO_2 gas:
The average volume of dissolved CO_2 gas (Table 1) was 6.04 ml for Sprite (SP_1-SP_3) and 7.87 ml for Mirinda samples (MIR_1 –MIR_3).

Table 1.Estimation of the volume of dissolved CO_2 gas.

Sample (n=3)	Volume of the sample after degassing (ml), Mean ± SD	Volume of dissolved gas (ml), Mean ± SD	RSD (%) of dissolved CO_2 gas (ml)
Sprite	94.03 ± 0.15	5.97 ± 0.15	0.16
Mirinda	95.17 ± 0.06	4.83 ± 0.06	1.19

3.2 Physico – chemical characteristic:

Physico chemical characteristic of the sample were evaluated and results presented in table.2. The average residue content was 11.1g/100 ml of Sprite and 13.36 g/100 ml for Mirinda samples, respectively. Both samples is acidity nature.

Table-2. Physico – chemical characteristic:

Sample (n=3)	pH (as such) Mean ± SD	Specific gravity @ 25 °C Mean ± SD	Residue on evaporation (%) Mean ± SD	Total acidity (as citric acid) Mean ± SD
Sprite	2.99 ± 0.02	1.0243 ± 0.01	11.1 ± 0.20	0.13 ± 0.01
Mirinda	2.53 ± 0.01	1.0258 ± 0.01	13.36 ± 0.15	0.17 ± 0.01

3.3 Linearity

Calibration curves were drawn by plotting concentration vs absorption and limit of detections were found out. Linearity of the curves was validated by the value of correlation coefficients (r^2 = 0.9985) shown in Fig. 1

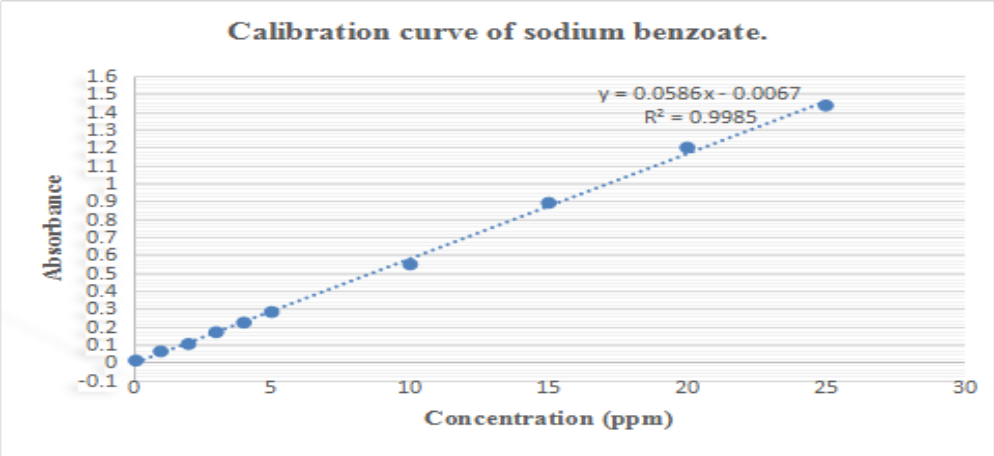

Fig.1 Calibration curve of sodium benzoate

3.4 Quantification of sodium benzoate and limit of detection:

The cleaned-up extracts of all the samples of Sprite (SP_1-SP_3) and Mirinda (MIR_1 –MIR_3) were found to give maximum absorption at 225 nm for sodium benzoate (Fig.3a- c). Limit of detection of sodium benzoate 0.05 µg/ml and limit of quantification of sodium benzoate 1.0 ppm (Figure 3d) showed sensitivity of the improved method. The amount of sodium benzoate (Table 2) in Sprite (SP_1-SP_3) and Mirinda (MIR_1 –MIR_3) samples results presented in table.2.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 [29]

Table 3. Amount of sodium benzoate content in sample.

Sample (n=3)	Total amount of sodium benzoate (µg/ml), Mean ± SD	Total amount of iron (µg/ml), Mean	Total amount of copper (µg/ml), Mean
Sprite	172.35 ± 3.29	≤ 1.0	≤ 1.0
Mirinda	396.44 ± 2.37	≤ 1.0	≤ 1.0

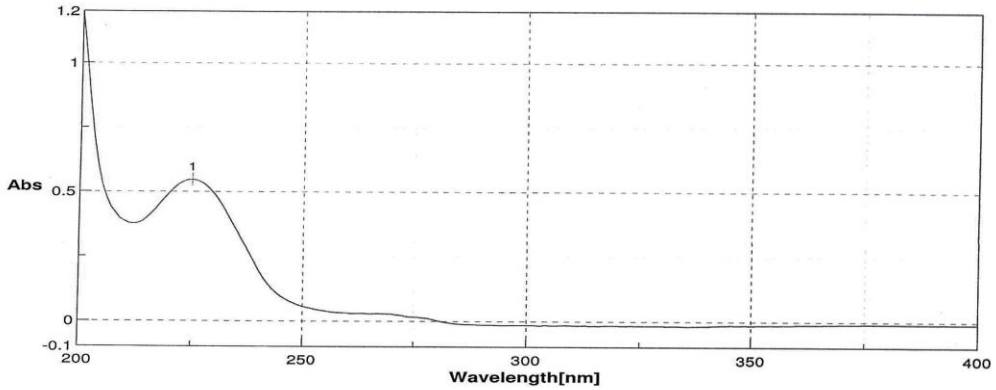

Fig.3 (a) UV spectrum of standard sodium benzoate,

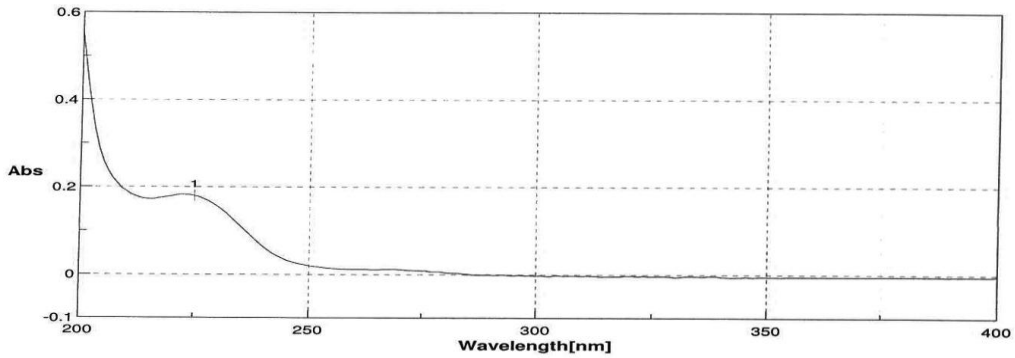

Fig.3 (b) UV spectrum of dilute sprite

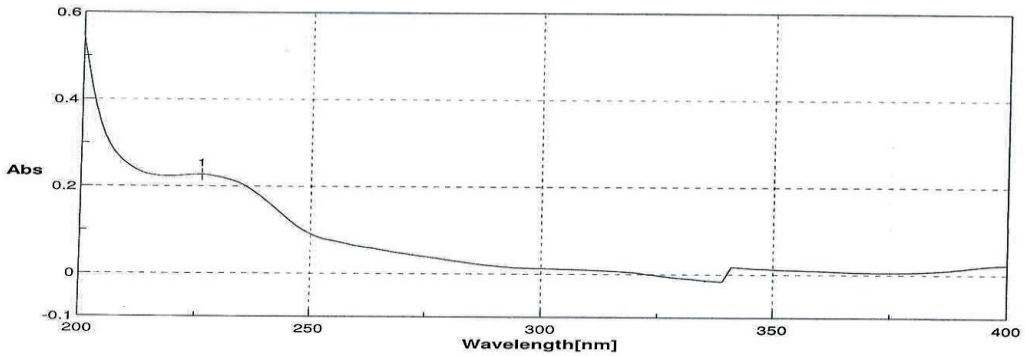

Fig. 3 (c) UV spectrum of dilute Mirinta.

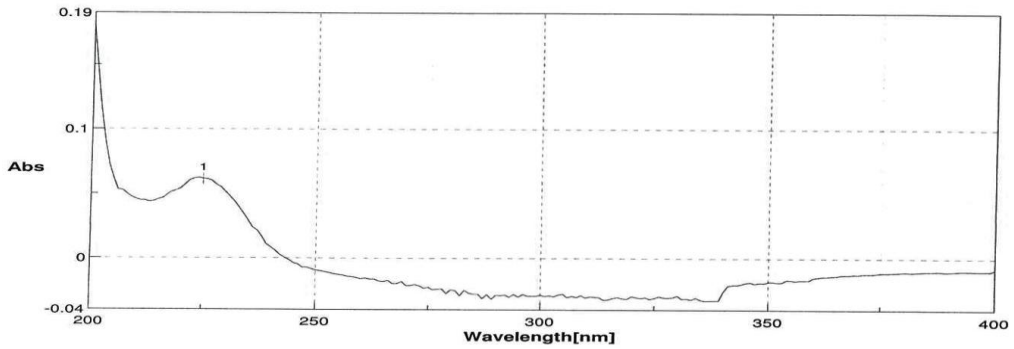

Fig. 3 (d) UV spectrum of LOQ level standard sodium benzoate

3.5 Accuracy

Recovery of sodium benzoate from dilute sample was determined at 1.0 µg/ml level concentrations. Average recovery was 87.7.0 % for sodium benzoate (Table 4, Fig.4)

Table 4. Results of recovery experiment.

Sample	Sample dilution (ml)	Absorbance value	Amount of sodium benzoate (ppm)	Absorbance value in after spiked sample	Amount of sodium benzoate (ppm) in after spiked sampe	Mean Recovery (%)
Sprite	0.01	0.07937	1.46859	0.1797	3.18079	87.7
	0.04	0.40432	7.01408	0.45844	7.93767	
	0.08	0.80372	13.8301	0.87632	15.06906	
	0.12	1.18044	20.2591	1.21267	20.8091	

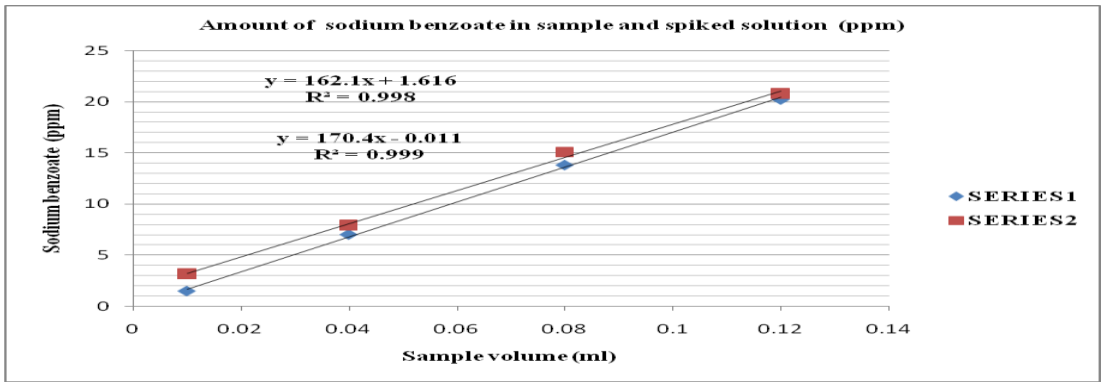

Fig. 4 Recovery of sodium benzoate

3.6 Effect of temperature and transition metal:

The effect of heat treatment about 90 °C in mixture of standard sodium benzoate and vitamin C in absence of transition metal (Fe or Cu), decrease vitamin C (Citric acid or Ascorbic acid) concentration observed using UV spectrum than without heat treatment of standard solution. Sodium benzoate reacts with citric acid or ascorbic acid to form benzene. So many literature reviews on benzene form in foods and soft drinks (Figure 5a-d).

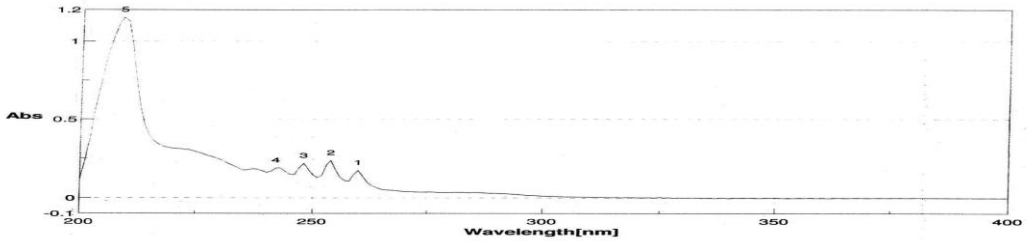

Figure 5 (a) UV spectrum of standard Benzene

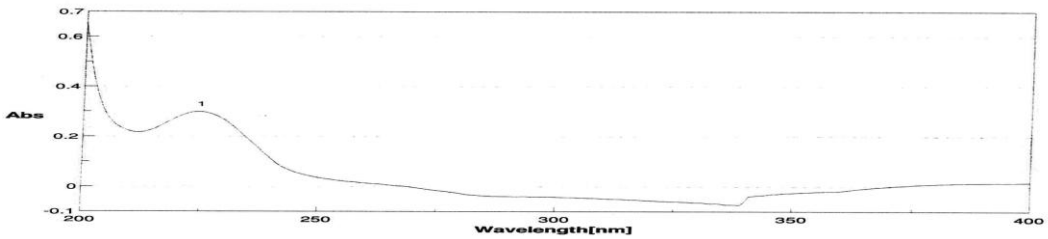

Fig.5 (b) UV spectrum of mixture of standard sodium benzoate and Citric acid

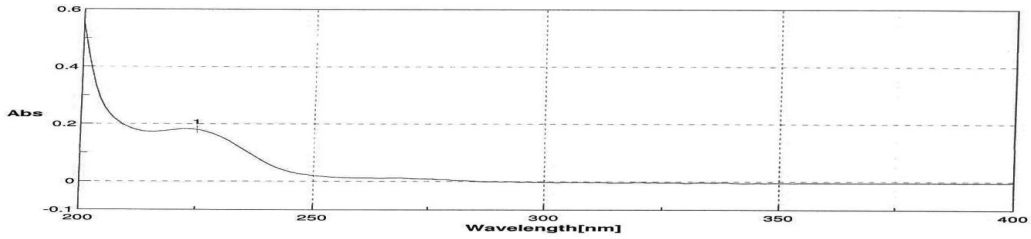

Fig.5 (c) UV spectrum of heat treatment sample for dilute Sprite

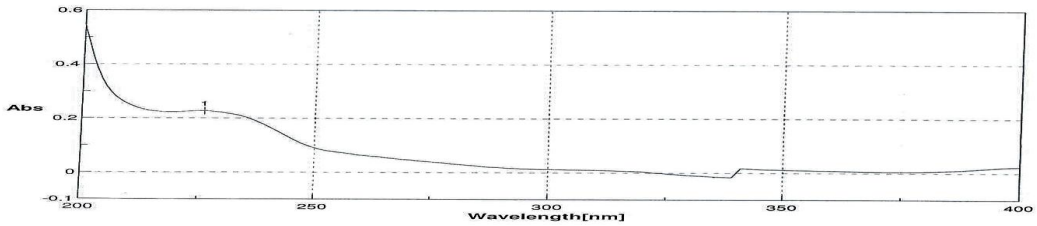

Fig.5 (d) UV spectrum of heat treatment sample for dilute Mirinta

IV. Discussion

From the study of six samples with two different carbonated drinks Sprite and Mirinda, Carbon dioxide gas dissolved in soft drinks under pressure with dissolved gas was higher in Sprite, when compared to Mirinda. The physico-chemical property characterized, sample is acidity in nature, due to preservative which was active in acid medium and higher value of residual content in Mirinda indicated higher proportion of additives than Sprite. In order to obtain greater sensitivity, sodium benzoate was analyzed at their wavelength maximums of 225 nm.The best fit standard curves were prepared by linear regression of different concentrations. The curve showed linear range from 0.1 to 25 mg L^{-1} for sodium benzoate. Curves showed a 0.9998 correlation co-efficient (R^2). This method detection limit (MDL) and method quantification limit (MQL) for sodium benzoate was 0.02 and 0.05 mg L^{-1}. All these samples were analyzed for sodium benzoate content and the results of sodium benzoate in 172.35 ± 3.29 µg/ml in Sprite and 396.44 ± 2.37 µg/ml in Mirinda. The recovery studies performed which found to be 87.7% for sodium benzoate after spiking with standard solution 1.0 mg L^{-1}. The study of soft drinks samples, average quantity of sodium benzoate was in the range of 181 to 191 µg/ml in Sprite and 140 to 160 µg/ml in 7up (lemon) samples [30]. Current work supports thermal stability where preservative content was almost stable but vitamin C content decreased with increase in temperature, which helps to establish processing and storage conditions. The samples evaluated in formation of benzene was negative. Gardner and Lawrence (1990) showed that hydroxyl radicals generated by the reduction of oxygen or hydrogen peroxyde by ascorbic acid, catalyzed by metal ions, could decarboxylate benzoic acid through a pH-dependent reaction, resulting in the benzene formation in foods and beverages [25]. The limit 10 ppb established by the World Health Organization, 5 ppb by the United States Environmental Protection Agency, and 1.0 ppb by the European Council [31 - 32]. In this method is very much easy to carry out and also cheaper than other analytical methods such as HPLC, Gas chromatography-Mass Spectrometry [16-21].

V. Conclusion

The illustrated UV-Vis spectrophotometric method for the determination of sodium benzoate is satisfactory for a minimum 0.05 µg/ml concentration. They study revealed that the preservative contained in soft drinks sample within Food and Drug Administration limit [13]. This method used also find out benzene in the soft drinks up to 1.0 ppm. It can be concluded that the method is sensitive and reproducible for routine analysis of sodium benzoate in soft drinks within a short period of analysis.

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