

Development of a Qualitative and Quantitative Method for Determination of Artificial Food Colourants in Carbonated Beverages

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ABSTRACT: Artificial food colourants play a significant role in the food industry as a food additive, because of their desired ecstatic quality made for the food item by increasing the attractiveness and marketability. Though qualitative tests have been used to identify food colourants, quantification is not often practiced. This study was carried out to develop a quantitative method for determination of artificial food colourants present in carbonated beverages. In this study, sheep wool is used as solid phase extractant (SPE) for the isolation and pre-concentration of artificial colourants present in foods. Preparative thin layer chromatography (TLC) is used for separation of food colourants as most soft drinks contain several colourants. UV-Visible spectrophotometry is employed for identification and quantification of separated food colourants. Using the results obtained, it can be concluded that the developed method is adequate for the purpose.

KEYWORDS: Artificial food colourants, Carbonated soft drinks, Solid phase extraction, Preparative Thin Layer Chromatography, UV-Visible spectrophotometry

I. INTRODUCTION

Colour is the first sensory parameter which gives a perception about a food or drink. Food colourants are the type of food additives which is responsible for the colour of the food or drink which can be further categorized as artificial food colourants and natural food colourants.

Artificial food colourants play an important role over natural food colourants because they impart bright, clear and uniform colours even with a small quantity, the colourants are stable being less sensitive to oxidation, heat, light, pH, etc and they can be used in combinations with each other to obtain different colour effects. They have a good water solubility which makes them compatible with many types of food and beverages. Water solubility can be reasoned due to the presence of a hydrophilic part along with a hydrophobic part and a conjugated chromophore.

According to Miniotti *et al.*¹, artificial food colourants have been categorized into five classes. They are the azo compounds, the triarylmethane group, the chinophthalon derivative of Quinoline Yellow, xanthenes and indigo colourants. Tartrazine (E 102), Sunset Yellow FCF (E 110), Azorubine (E 122), Amaranth (E 123) and Ponceau 4R (E 124) are some of the examples of azo compounds. Brilliant Blue FCF (E 133) and Green S (E 142) falls under the triarylmethane group while Quinoline Yellow (E 104) comes under chinophthalon derivative of Quinoline Yellow. Erythrosine (E 127) is a xanthene and Indigo carmine (E 132) is an indigo colourant.

Even though synthetic colourants have these advantages over natural colourants, they have a potential risk to cause adverse health effects. They are susceptible to be carcinogenic and toxic. It is reported by de Andrade *et al.*², that they can lead to diseases linked to allergies such as skin rash and bronchial asthma in susceptible people.

Hyperactivity in children is also considered as an adverse effect of the consumption of artificial food colourants. Therefore, according to Altinoz *et al.*³, as with many other food additives, the analytical control of these dyes is of considerable importance.

As every country, Sri Lanka has a set of permitted artificial food colourants which are considered to be less harmful than the other artificial food colourants. According to the Food (Colouring Substances) Regulations – 2006 and its

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amendments, the synthetic colourants that are permitted to be used in food are given in the Table 1. The food regulation states that the synthetic food colourants should have a minimum of 85.0% pure dye. The pure dye should contain water insoluble matter less than 0.1%, subsidiary dye less than 4.0%, ether extractable matter less than 0.2% and intermediates less than 0.5%.

TABLE 1
PERMITTED ARTIFICIAL FOOD COLOURANTS IN SRI LANKA

Artificial food colourant	INS number
Carmoisine	122
Ponceau 4R	124
Erythrosine	127
Allura red	129
Sunset Yellow FCF	110
Tartrazine	102
Indigotine	132
Brilliant Blue FCF	133
Fast Green FCF	143

Carbonated drinks come under ready-to-drink category of soft drinks and among these carbonated beverages, there are several types which are colourless while the others are colourful. The majority of the coloured beverages contain synthetic food colourants alone or in combination with natural food colourants. Among all other types of soft drinks, carbonated drinks are dominating in the world market due to its convenience in consumption. Therefore, it is important to develop an analytical method to detect and quantify the artificial food colourants in them.

To avoid the interference of other ingredients in the medium, food colourants have to be isolated as the first step of the analysis. Adsorption of food colourants onto sheep wool is a commonly used technique in food analysis because wool protein is a suitable binding agent for this purpose due to its insolubility and the ability of manipulating its charge by changing the pH, because at low pH values wool proteins get positively charged due to protonation. Usually the artificial food colourants are negatively charged and therefore they form electrostatic bonds making colourants get extracted into the wool.⁴ This result can be reversed by making the pH of the medium high. Even though many studies have used solid phase extraction of colourants into sheep wool for qualitative analysis, it is not used as a quantitative technique frequently. Gilhooley *et al.*⁵ and Hofer *et al.*⁶ have reported that extraction of food colourants into sheep wool is not suitable for quantitative studies.

The principal problem associated with the determination of colour additives as the simultaneous determination of two or more compounds in a sample without prior separation of the components. Chromatography is frequently used as an analytical technique for separating a mixture of chemical substances into its individual components, so that the individual components can be thoroughly analyzed. Thin layer chromatography (TLC) is the technique where a glass plate coated with a thin layer of silica on which is spotted the compound is the stationary phase and a suitable solvent system is used as the liquid mobile phase. Depending on the polarity of the individual artificial food colourant, its affinity to the stationary phase varies. Therefore, as silica induces a polarity to the stationary phase, more polar colourants will get attracted more and the less polar colourants with less affinity will be eluted first, with the flow of a suitable mobile phase. According to Farzianpouret *al.*⁷, the solvent system n-butanol, distilled water and acetic acid (20:12:5) has been used as the mobile phase for preparative TLC. It is reported by Mohamed *et al.*¹¹ that among solvent systems n-butanol: absolute ethanol: 2N ammonium hydroxide (60:20:20) and iso amyl alcohol: glacial acetic acid: water (40:20:20), the solvent system n-butanol: absolute ethanol: 2N ammonium hydroxide (60:20:20) was better resolving than the other.

UV-visible spectrophotometry which is widely used in many applications can be used in the qualitative and quantitative analysis of artificial food colourants because each food colourant has a specific wavelength value which is corresponding to its maximum absorption of light.

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This study was carried out to develop a method to identify and quantify the artificial food colourants present in carbonated beverages, using three consecutive steps, namely, solid phase extraction of food colourants into sheep wool, separation of food colourants into individual components by chromatography and detection and quantification by UV-Visible spectrophotometry.

II. MATERIALS AND METHODOLOGY

a. Materials

Powders of food colourants (Carmoisine, Ponceau 4R, Tartrazine, Sunset Yellow FCF, Brilliant Blue FCF)
Glacial acetic acid
Dilute acetic acid
10% ammonium hydroxide
2N ammonium hydroxide
Absolute ethanol
n-Butanol
Preparative TLC plates (20 cm × 20 cm glass plate coated with silica of layer thickness 250 μm)
White colour 100% sheep wool
Carbonated beverages

b. Methodology

1. Development of the analysis method

For the analysis, 5 types of carbonated drinks from the Sri Lankan market were selected such a way that all 5 types contain only artificial food colourants. From each type of drink, 15 samples were selected and they represented 3 batches, so that 5 samples were from one particular batch.

Out of 5 types, 3 types were composed of single artificial food colourant while 2 types were composed of a combination of artificial food colourants.

TABLE 2
TYPES OF FOOD COLOURANTS INCLUDED IN THE 5 TYPES OF CARBONATED DRINKS

Artificial food colourant	Carbonated drink				
	A	B	C	D	E
Carmoisine (E 122)					
Ponceau 4R (E 124)					
Tartrazine (E 102)					
Sunset Yellow FCF (E 110)					
Brilliant Blue FCF (E 133)					

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Therefore, for the experiments carried out in this section, solutions of food colourants were prepared by combining food colourants that corresponds to each type of carbonated drink.

1.1. Extraction of food colourants into sheep wool

To the solution containing synthetic food colourants, pieces of white coloured 100% sheep wool which are about 10 cm in length were added. Then the medium was acidified with 10 ml of dilute acetic acid and the solution was placed on a hot water bath for the extraction of colourants. After the solution turned colourless, the pieces of sheep wool were removed from the medium and another piece of white sheep wool was added to adsorb any colouring matter remaining in the medium. Then all pieces of sheep wool were washed well with deionized water. Then they were dried by placing in an oven at 105°C for five minutes.

1.2. Elution of food colourants from sheep wool

The sheep wool with extracted food colourants were placed in a clean glass beaker and a portion of 10% ammonium hydroxide solution was added. Then the beaker was placed on a hot water bath and heated till the adsorbed colourants get eluted to the aqueous solution. The aqueous solution was decanted into a glass evaporating dish. Then it was added with another portion of 10% ammonium hydroxide and the same procedure was carried out. This was continued till all the adsorbed synthetic colourants get eluted from the sheep wool and the sheep wool turned into its original colour. Finally heating of the aqueous solution of colourants was continued till a residue remains at the bottom of the dish. Residue of solutions corresponding to carbonated drinks with single food colourants were made into 50.0 ml solutions and the others were made into 25.0 ml solutions

1.3. Separation of food colourants into individual components

Preparative TLC was used as the separation technique and it was carried out for the solutions with multiple food colourants.

Preparative TLC plates were placed in an oven; preheated to 105°C for 30 minutes for the activation and allowed to cool back to the room temperature. Then extracted colourants were spotted as bands on activated preparative TLC plates.

Meanwhile, the solvent tank was prepared by allowing it to get saturated with the vapour of the solvent system. As the solvent system, the best resolving solvent system between n-butanol, distilled water and acetic acid (20:12:5) system and n-butanol: absolute ethanol: 2N ammonium hydroxide (60:20:20) system was selected.

A volume of 200 µl of the solutions of food colourants made from the residue of the colourants extracted into sheep wool, were spotted as a band in the preparative TLC plate and placed in the solvent tank for separation to occur. Then the bands of colourants separated into individual components were scraped using a spatula and were transferred into clean dried beakers separately. Then colourants were eluted from silica using deionized water as the polar solvent. The filtrates were evaporated on a hot water bath and the obtained residues were dissolved in accurately measured volumes of deionized water separately.

1.4. Detection and Quantification

The absorption spectra of the original solutions of artificial food colourants were obtained using the UV-Visible spectrophotometer (Shimadzu UV-1800 series) assisted by the UVProbe software (2.43 version). The wavelength of each colourant which gives the maximum absorption in the visible region (λ_{max}) was determined using the above obtained absorption spectra.

Then with proper dilutions of the original solutions of artificial food colourants, a series of standard solutions of colourants were prepared for every colourant such that their absorbance lies in the range of 0.2-0.8. These standard series of concentrations were utilized to develop calibration plots for the colourants in the UV-Visible spectrophotometer and the developed calibration plots were used to determine the concentrations of the food colourants. The concentration of food colourants corresponding to carbonated drinks with a single food colourant were

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determined using the 50.0 ml solution prepared in the section 1.2 and solutions with multiple food colourants were analyzed using the final solutions of individual artificial food colourant resulted in the section 1.3.

2. Analysis of carbonated beverages

Colourants in 350.00 ml of each carbonated drink were analyzed proceeding through the steps given in the section 1 of methodology.

3. Recovery study

From each type of carbonated drink having only one food colourant, two samples were obtained from the same batch. From the degassed drink of one sample 350.00 ml was transferred into a 500 ml conical flask.

To another 500 ml flask, 175.00 ml of the other sample was added after degassing. Then a volume of 175.00 ml of a solution of synthetic food colourants with a known concentration was added. There the solution of colourants was the same colourant or mixture of colourants that the particular type of carbonated drink was containing. Then the same procedure as in the section 1 was carried out. There the concentrations of two samples of a particular type of carbonated drink were assumed to be equal as they were obtained from the same batch.

III. EXPERIMENTAL RESULTS

Absorption spectra of food colourants obtained from the UV-Visible spectrophotometer were plotted on the same graph. According to the Figure 1, the wavelengths of each colourant at which the colourants show maximum absorption in the visible region were determined.

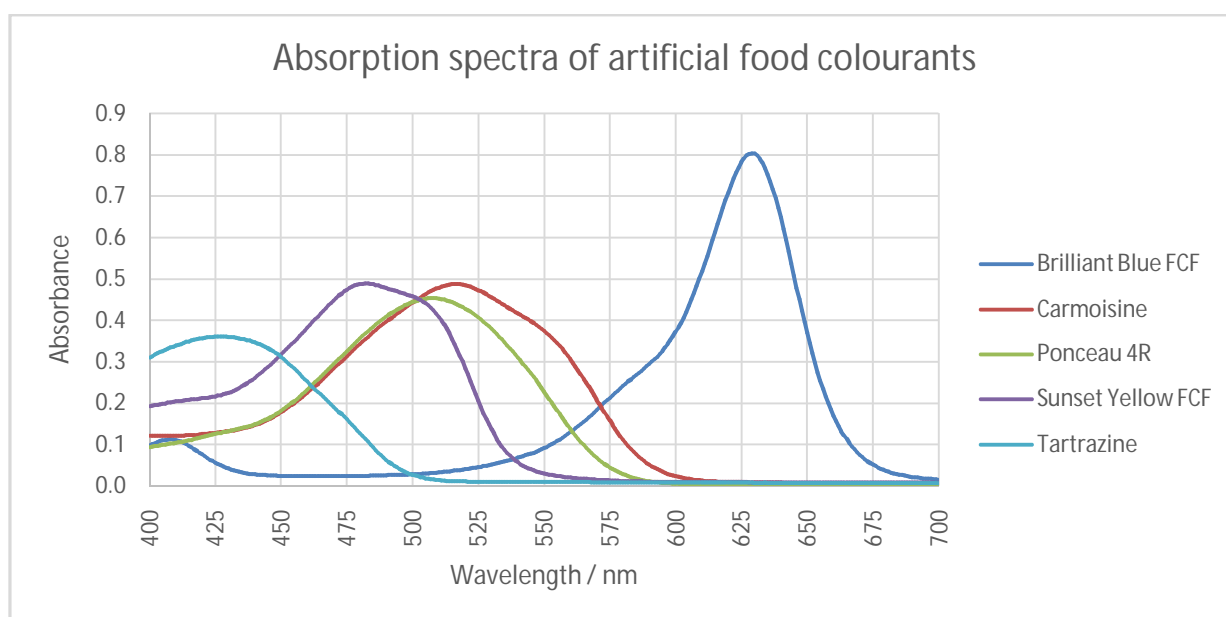


Figure 1: Absorption spectra of artificial food colourants

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TABLE 3
WAVELENGTHS WITH MAXIMUM ABSORPTION OF THE COLOURANTS

Artificial food colourant	λ_{max} / nm
Tartrazine	426
Sunset Yellow FCF	482
Ponceau 4R	508
Carmoisine	516
Brilliant Blue FCF	629

The solvent systems n-butanol, distilled water and acetic acid (20:12:5) and n-butanol: absolute ethanol: 2N ammonium hydroxide (60:20:20) were used as the mobile phase in preparative TLC. By observing the separation of food colourants by each solvent system, it was decided to use the solvent system n-butanol: absolute ethanol: 2N ammonium hydroxide (60:20:20) as the mobile phase for the further analysis in the method development and the analysis of food colourants in carbonated beverages.

For the development of the method, the solutions of colourants made corresponding to each carbonated drink were analyzed. The concentrations given in the Table 4 were obtained. By this the accuracy of the method was also calculated.

TABLE 4
COMPARISON OF INITIAL CONCENTRATION WITH THE MEASURED CONCENTRATION

Food colourant	Initial concentration / ppm	Measured concentration / ppm	% Error
Tartrazine	20.00	18.81	5.95
Sunset Yellow FCF	20.00	18.82	5.95
Ponceau 4R	20.00	18.96	5.20
Carmoisine	20.00	19.32	3.40
Brilliant Blue FCF	20.00	17.56	12.20

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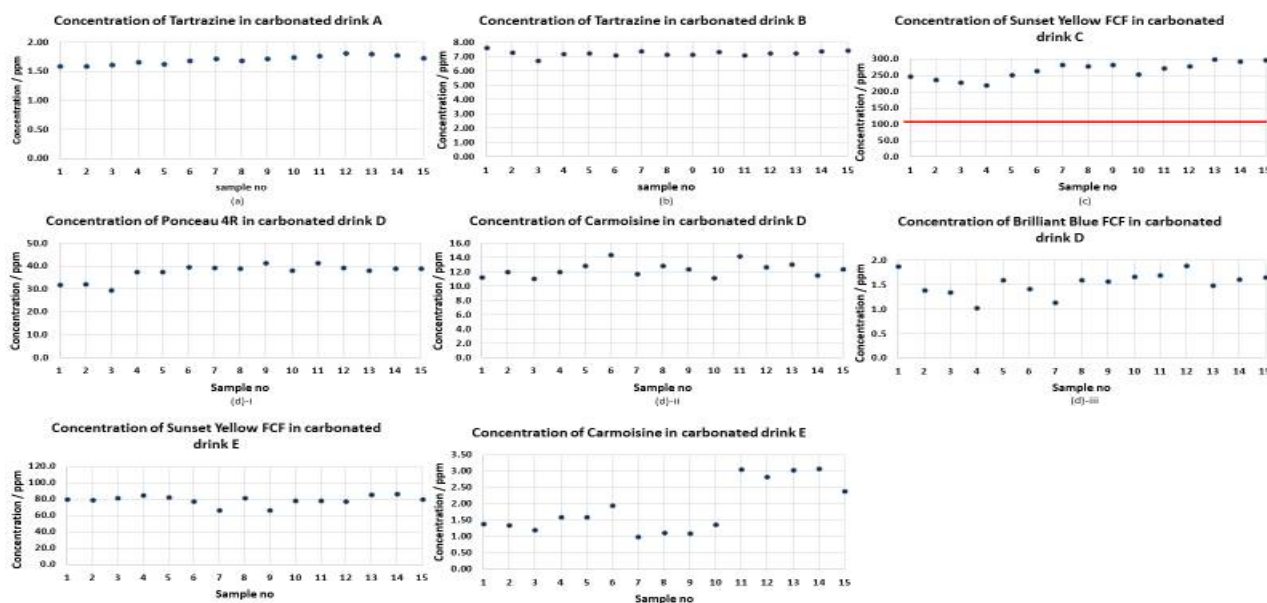


Figure 2: Results of the analysis of the artificial food colourants in carbonated drinks (a) Tartrazine in carbonated drink A (b) Tartrazine in carbonated drink B (c) Sunset Yellow FCF in carbonated drink C (d-i) Ponceau 4R in carbonated drink D (d-ii) Carmoisine in carbonated drink D (d-iii) Brilliant Blue FCF in carbonated drink D (e-i) Sunset Yellow FCF in carbonated drink E (e-ii) Carmoisine in carbonated drink E

The results of the analysis of 5 types of carbonated drinks can be summarized as given in the Figure 2. As every country Sri Lanka has a set of permitted food colourants according to the Food (Colouring Substances) Regulations – 2006 and its amendments. According to this regulation, the maximum permissible level of artificial food colourants that can be included in a particular type of food or drink is 100 ppm. Therefore, it can be concluded that all other 4 types of carbonated drinks except the carbonated drink C did not exceed the maximum permissible level.



Figure 3: (a) Carbonated drink before extraction of food colourants (b) Carbonated drink after the extraction of food colourants (c) Extracted colourants spotted on the preparative TLC place (d) Developed preparative TLC plate (e) Solutions of separated colourants

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A recovery study was carried out to check the adequacy of the developed method in determining the concentrations of artificial food colourants in carbonated drinks. Here two samples from the same batch were selected, so that their concentrations of colourants were assumed to be equal. According to the obtained results given in the Table 5, the method can be concluded as a suitable analytical method to quantify artificial food colourants in carbonated beverages with high recovery.

TABLE 5
RECOVERY STUDY

Carbonated drink	Artificial food colourant	Initial concentration / ppm	Measured concentration / ppm	Recovery percentage / %
A	Tartrazine	10.00	9.71	97.1
B	Tartrazine	10.00	9.45	94.5
C	Sunset Yellow FCF	100.00	93.88	93.9
D	Ponceau 4R	10.00	8.94	89.4
	Carmoisine	10.00	9.13	91.3
	Brilliant Blue FCF	10.00	9.41	94.1
E	Carmoisine	10.00	8.82	88.2
	Sunset Yellow FCF	10.00	9.26	92.6

IV. CONCLUSION

This investigation was carried out to develop a method to detect and quantify the synthetic food colourants added in carbonated beverages. According to the recovery study carried out to check the adequacy of the developed method, very high percentages of recovery were obtained. Adsorption on wool as the isolation technique has been preferred only for qualitative studies. But the calculated percentages of recovery in this study implies that adsorption on wool can be utilized for quantitative studies also.

According to the Food (Colouring Substances) Regulations- 2006 and its amendments, the addition of the permitted synthetic colouring substances to a food or beverage ready for consumption, shall not exceed 100 ppm as a single component or in combination. But according to the results obtained in this study, it is clear that some types of carbonated drinks available in the Sri Lankan market exceed this safety limit.

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