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# CONTROL OF THE SYNTHETIC COLORANTS IN CARBONATED SOFT DRINKS IN THE LOCAL MARKET

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**ABSTRACT**

Synthetic colorants are commonly used in soft drinks. Since synthetic colorants can cause several adverse side effects, study and make them come under control is very important. Identification and quantification of three permitted synthetic colorants (Sunset Yellow, Tartrazine and Brilliant Blue FCF) and determination of any potential banned colorants in some soft drinks samples in the Syrian local market was carried out in this work. The results obtained showed that column chromatography, wool dyeing and thin layer chromatography (TLC) methods were precise to determine the synthetic colorants added either individually or in binary combination. The whole samples are considered to be safe for consumption since they were contained permitted colorants at a concentration lower than the max permitted level in Syrian standards.

## INTRODUCTION

Food coloring, or color additive, is any dye, pigment or substance that imparts color when it is added to food or drink. They come in many forms consisting in liquids, powders, gels and pastes [1]. Artificial colors are added to food products that are aesthetically and psychologically attractive[2] to enhance their taste, to promote sales [3] to influence consumer purchasing decisions [4]and to provide color to colorless and "fun" foods [5]. People use color to identify a food and to predetermine its quality [6]. Synthetic colorants are a very important class of food additives. However some of these substances are potentially risky to human health, especially if they are excessively consumed [7]. The use of non-permitted colors is known to cause

adverse effects in experimental animals and in humans. Repeated exposure to even the permitted synthetic colors may be hazardous) [8]. To protect public health, many countries have established strict regulations for the allowable kinds and concentrations of dyes [9]. In Syria the use of colorants is organized by the national standards No.770-2011 which include synthetic, natural and nature identical food colors that are permitted to consume by humans at the recommended intakes. Thus, there are various methods have been proposed to detect the colorants in food and soft drink samples such as High Performance Liquid chromatography-HPLC [10,11], capillary electrophoresis-CE [3-12], differential pulse polarography [13], differential pulse voltammetry method (Medeiros et al, 2012)14, cyclic and differential pulse voltammetry method [15], spectrophotometric method [16,17], chemometric method followed by spectrophotometric method [18], derivative spectrophotometric method [19-20], Thin Layer Chromatography-TLC[21,22]. Tartrazine(TAR) and Sunset yellow(SY) are two synthetic azo dyes among the widest additives used in food and especially in soft drinks. TAR and SY are added either individually or in binary combination In order to realize all diverse yellow-shades to different food, drinks and drugs [23]. Brilliant Blue FCF(BB) is a triarylmethane dye and can be used with Tartrazine to produce various green shades[14,24], fig.1. SUN, TAR and BB are permitted synthetic colorants with maximum permitted level 100mg/L in soft drinks according to Syrian national standards.[25-26]

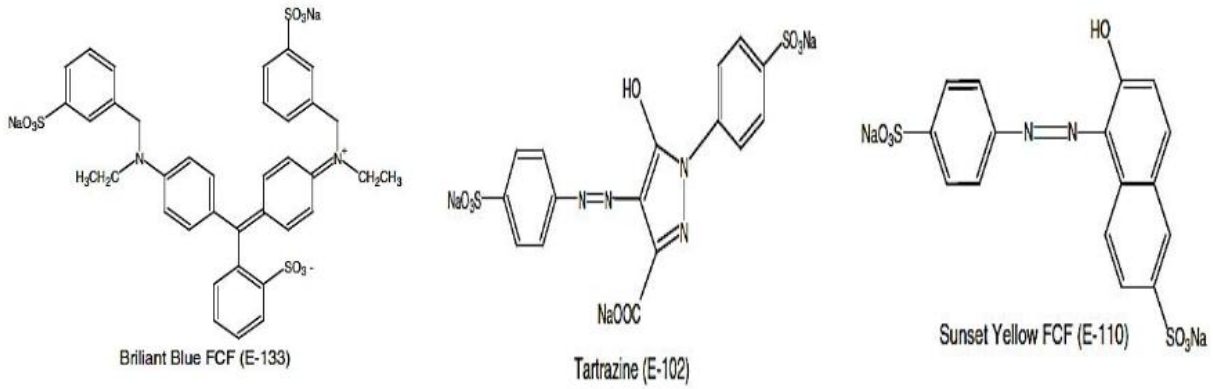


**Figure 1 Structural formula of Sunset yellow, Brilliant Blue, Tartrazine**

The aim of this study was to quantitatively and qualitatively detect three common colorants TAR, SY, BB and any potential banned colorants [27] in the local soft drink samples. Different analytical techniques were used such as Wool dyeing, Column chromatography followed by spectrophotometric method to determine Sunset yellow content in the samples with *single synthetic* food *colorant*. Samples that contained synthetic colorants in binary combination were analyzed by TLC (Thin layer chromatography) method to determine the type of colorants and their concentrations. [28,29,30]

A total of 48 soft drink samples containing synthetic colorants were collected during 2014-2015. Four brand samples of carbonated soft drinks were categorized as A, B, C, D each category samples were of 2 different qualities. A and B brand Samples contained Sunset yellow as a labeled colorant. C brand Samples contained combined Sunset yellow and Tartrazine as labeled colorants. D brand Samples contained combined Tartrazine and Brilliant Blue FCF as labeled colorants. The samples were purchased from Syrian local markets.Table,1.

## 2. MATERIALS AND METHODS

### 2.1. SAMPLES

**Table.1: Fizzy drink samples**

| Soft drink brand | Flavor | Labeled colorants | Number of samples |
|---|---|---|---|
| **A** | Orange | SY | 12(6 for each lot) |
| **B** | Orange | SY | 12(6 for each lot) |
| **C** | Orange | SY+TAR | 12(6 for each lot) |
| **D** | Green apple | TAR+BB | 12(6 for each lot) |

### 2.2. CHEMICALS AND APPARATUS

- Standard synthetic colorants(Sunset yellow, Tartrazine, Brilliant Blue FCF),
- Hydrochloric acid(CHEMLAB),
- Ammonia(BDH),
- Methanol(BDH),
- Butanol (TEKKIM),
- Aluminum oxide(BDH),
- Glacial acetic acid(BDH),
- Distilled water,
- Chromatographic column(2,1×45 cm),
- white wool,
- silica gel plates(Macherey-Nagel),

- micropipette(10 l),
- capillary tubes,
- solvent tank,
- ultrasonic bath,
- UV-VIS spectrophotometer( Jasco v-530 UV).

## 2.3. STANDARD SOLUTIONS

Individually, a standard stock solution containing Tartrazine1-3 mg/100 ml , Sunset Yellow1-3 mg/100 ml, Brilliant Blue FCF 1 - 3 g/ml, was prepared by dissolving 100 mg standard colorant in 100mL hydrochloric acid 0.1N. Each working standard solution of colorants was prepared by appropriate dilution of 100 mg of standard colorant in 100mL hydrochloric acid 0,1N to obtain the concentration range for each standard as mentioned above taking into consideration the purity of the colorants. The wavelength of peak absorbance for each of these dyes was achieved at wavelength of 482nm for Sunset Yellow, 427nm for Tartrazine and 630nm for Brilliant Blue FCF.

## 2.4. METHODS

### 2.4.1. SAMPLE PREPARATION

Samples of fizzy drinks were previously degassed for 15 min in ultrasonic bath and directly analyzed without any dilution.

### 2.4.2. WOOL DYEING

An appropriate volume 5-10 ml of the sample was taken in beaker and acidified with 1% hydrochloric acid. Then, a piece of white wool was immersed in the solution that was boiled for 60 minutes using water bath. After the wool has taken up the color, it was removed from the beaker, washed with distilled water. The wool was transferred to a beaker and the color was stripped from the dyed wool by boiling in a dilute solution of ammonia (1 part of strong ammonia to 50 ml of water). The used wool was discarded and the colored solution was concentrated by evaporation on water bath. After the removal of ammonia the colored solution was evaporated to a small volume for pure spotting. The colored concentrate was diluted with a small volume of 0,1N HCl and transferred to a 100 ml volumetric flask and the volume was made up the *volume* to *100ml* with 0,1 N HCl. the optical density was determined at different wavelength to obtain a plot of optical density versus wavelength. The wavelength corresponding to the maximum optical density was 482 nm for SY as determined from this plot, was noted and the wavelength was used for the preparation of the calibration curve used in the determination of Sun yellow concentration

### 2.4.3. COLUMN CHROMATOGRAPHY

An appropriate volume of the Sample was passed through a column(2,1 × 45 cm) containing aluminum oxide acidified with 1% HCl. The adsorbed color was eluted with 1% ammonia. The eluted then was evaporated to dryness on a hot water bath dissolved the residue in 0.1 N HCl, transferred quantitatively to a 100 ml volumetric

flask and made up to the volume with 0.1N HCl. The optical density was determined at different wavelength to obtain a plot of optical density versus wavelength. The wavelength corresponding to the maximum optical density was 482 nm for SUN as determined from this plot, was noted and the wavelength was used for the preparation of the calibration curve used in the determination of Sun yellow concentration.

### 2.4.4.TLC

The uptake of color is carried out by a wool-dyeing technique. The silica gel was firstly activated at 100° C for 4-8 minutes . Solutions of colorants as well as their references were spotted in the plate by means of capillary tubes. They were applied 3 cm from the bottom of the plate. Butanol, distilled water and glacial acetic acid (20:12:5) were used as a mobile phase. The stationary phase used was silica gel. The colorant spots from the plates were cut and eluted with methanol-water (1:1,v/v). The TLC plates were placed in the chromatographic chamber containing the appropriate solvent and the solvent was allowed to run for a certain distance from the base line. Plates were allowed for air drying and the Rf-values were calculated. Colorants identification was carried out by comparing the Rf values of the sample colorants with Rf values of standard colorants and by the absorption spectra with that of standard colorants. Colorants quantification was carried as described previously but the standard colorant solutions in hydrochloric acid (2.5 mg/100ml for SY and TAR, 1.5 g/ml for BB) and the sample colorant solutions were stained at 10 l/spot. The separated colorants in the studied samples were quantified as the colored spots were scratched from the sample and the colorants were extracted with a mixture of methanol-water (1:1, v/v) and analyzed through UV-VIS spectrophotometry

## 3 Results and Discussion

### 3.1Calibration curves

Linearity of calibration curve for HPLC method was checked on the basis of the chromatographic peak areas using fizzy drink samples fortified with various concentrations of mixed standard solutions Sun set yellow, Tartrazine and Brilliant Blue in a concentration range 0-100 mg/L. The calibration curve was linear for all the ranges of interest, with a good regression coefficient, of 0.9846 for SY and 0.9726for Tar and0.9918 for BB respectively as shown in Figs2, 3, 4 where Y is the absorption value and X is the concentration value (mg/100ml for SY and TAR, g/ml for BB).

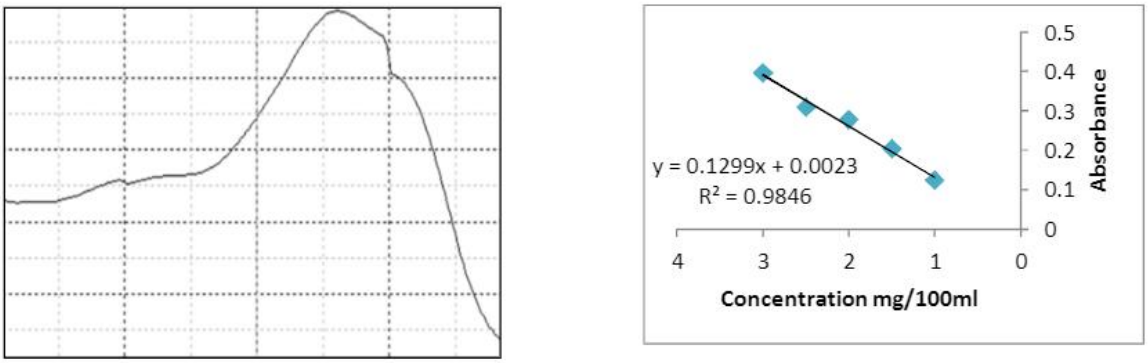


**Figure 2** Calibration curve and spectrum of SUN at 482 nm

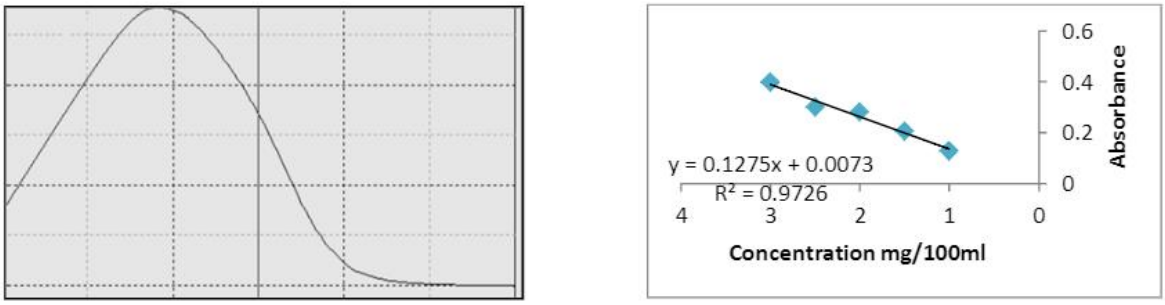


**Figure 3 Calibration curve and spectrum of TAR at 427 nm**

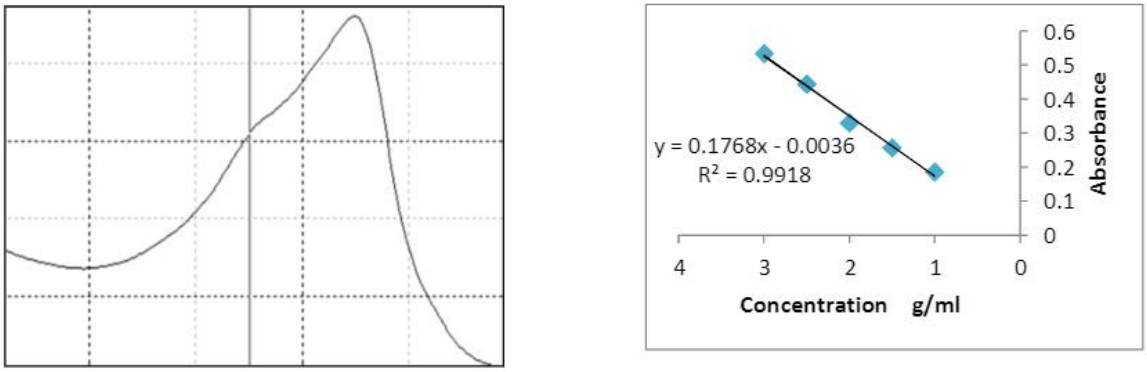


**Fig.4Calibration curve and spectrum of BB at 630 nm**

### 3.2. Comparative Sunset yellow determination using Wool dyeing and Column chromatography techniques

Comparing the spectra of the detected colorants in the studied sample and SY standard spectrum, it was found that A and B samples contained single added colorant as labeled on the product at a

concentration below the maximum level as set by Syrian standards. Wool dyeing technique showed that SY colorant was higher than it was detected by column chromatography as shown in Table 2,3.

**Table. 2: Colorant content ( mg/L) determined by column chromatography method**

| Fizzy drink product | Labeled Colorants | Determined Colorants | Lot | Mean Concentration mg/L | RSD% |
|---|---|---|---|---|---|
| A | SY | SY | 1 | 16.2457 | 1.0279 |
| | | | 2 | 16.1752 | 1.2284 |
| B | SY | SY | 1 | 9.8960 | 1.1782 |
| | | | 2 | 9.3584 | 1.6787 |

**Table. 3: Colorant content ( mg/L) products determined by Wool dyeing method**

| Fizzy drink Product | Labeled Colorants | Determined Colorants | Lot | Mean Concentration mg/L | RSD% |
|---|---|---|---|---|---|
| A | SY | SY | 1 | 18.9607 | 3.5647 |
| | | | 2 | 17.5493 | 2.0889 |
| B | SY | SY | 1 | 11.8218 | 2.8718 |
| | | | 2 | 11.3202 | 3.0485 |

To comparatively evaluate the recoveries between column chromatography and wool dyeing techniques, a spiked solution of SY colorant standard (0.25 mg/100 ml solution in 0.1 HCl) was prepared. Recoveries about 92% and 93% were determined based on column chromatography and wool dyeing respectively where Relative standard deviations were closer to 1% and 3% for column chromatography and wool dyeing respectively. Table 4.

**Table.4: Recoveries for column chromatography and wool dyeing methods**

| | | Column chromatography | Wool dyeing |
|---|---|---|---|
| | | Concentration mg/100ml | Concentration mg/100ml |
| Record | 1st record | 2.3156 | 2.3802 |
| | 2nd record | 2.3033 | 2.3687 |
| | 3rd record | 2.3548 | 2.3456 |
| | 4th record | 2.3102 | 2.3017 |
| | 5th record | 2.2771 | 2.4526 |
| | 6th record | 2.2848 | 2.2355 |
| Mean average | | 2.3076 | 2.3473 |
| Standard Deviation | | 0.0264 | 0.0728 |
| Relative Standard Deviation% | | 1.1440 | 3.1014 |
| Real value | | 2.5 | 2.5 |
| Recovery % | | 92.304 | 93892 |

## III. Binary combined colorants determination by TLC

Identification and quantification of synthetic colorants added to C (SY+TAR) and D(TAR+BB) products were determined by TLC technique. TLC based confirmation of colorants identity was carried out by spectral and Rf comparison where the Rf values of spots of sample's dye and of standard's dye were compared Table 5. Besides, after separation, spectra of sample's dye and that of standard dye were compared. Then, quantification of analytes was measured. The results showed that C samples contained two permitted synthetic colorants of SY and TAR, as well as D samples contained two permitted synthetic colorants of TAR and BB as labeled on the product's batch with concentration under the max permitted level in Syrian national standards, Table 6.

**Table. 5: Rf values of determined colorants in comparison with that of standard solutions of the studied colorants**

| Fizzy drink Product | Labeled Colorants | Detected Colorants | Rf for colorant in the studied samples | Rf for standard solutions of the studied colorants |
|---|---|---|---|---|
| C | SY | SUN | 0.7692 | 0.7094 |
| | TAR | TAR | 0.4786 | 0,4957 |
| D | TAR | TAR | 0.4552 | 0.4390 |
| | BB | BB | 0.8455 | 0.8292 |

**Table. 6: determination of Colorants content in C and D products using TLC technique**

| Fizzy drink product | Labeled Colorants | Detected Colorants | Mean Concentration mg/L | | RSD% | |
|---|---|---|---|---|---|---|
| | | | Lot 1 | Lot 2 | Lot 1 | Lot 2 |
| C | SUN | SUN | 12.5323 | 13.3481 | 1.9796 | 2.1785 |
| | TAR | TAR | 10.3198 | 9.9269 | 2.4196 | 2.5949 |
| D | TAR | TAR | 10.4041 | 9.5425 | 2.2654 | 2.2352 |
| | BB | BB | 0.7866 | 0.734 | 2.1993 | 2.3569 |

To evaluate the recoveries of TLC method a spiked solution of SY colorant standard (0.25 mg/100 ml solution in 0.1 HCl) was prepared and mixed with a spiked solution of TAR of 0.25 mg/100 ml solution in 0.1 HCl. The mixture was called C* solution. A spiked solution of BB was prepared at concentration of 1,5 g/ml in hydrochloric acid 0,1N and mixed with spiked solution of TAR prepared at concentration of 2,5 mg/100 ml in hydrochloric acid 0,1N and called D* solution.

The percent Recoveries and Relative standard deviations for SY and TAR in $C^*$ were 94% with RSD of 1.4 and 93% with RSD of 1.5 respectively for TLC technique. The percent Recoveries and Relative standard deviations for BB and TAR in D* were 92,2% with RSD of 2 and 92,8% with

RSD of 2.2 respectively for TLC technique (Table 7).

**Table.7: Recoveries determined using TLC technique**

| | | TLC | | TLC | |
|---|---|---|---|---|---|
| | | C* | | D* | |
| | | SY Content mg/100ml | TAR Content mg/100ml | BB Content mg/100ml | TAR Content mg/100ml |
| Record | 1st record | 2.3382 | 2.3903 | 1.3979 | 2.3779 |
| | 2nd record | 2.3333 | 2.3355 | 1.3490 | 2.2717 |
| | 3rd record | 2.3997 | 2.3164 | 1.4049 | 2.3165 |
| Mean average | | 2.3570 | 2.3474 | 1.3839 | 2.3220 |
| Standard Deviation | | 0.0346 | 0.0374 | 0.0282 | 0.0529 |
| Relative Standard Deviation% | | 1.4679 | 1.5932 | 2.0377 | 2.2782 |
| Real value | | 2.5 | 2.5 | 1.5 | 2.5 |
| Recovery% | | 94.28 | 93.896 | 92.26 | 92.88 |

Thus, the findings suggest that all studied samples of fizzy drink can be considered safe for consumption because they were contained permitted synthetic dyes at concentration lower than the max levels which were set in Syrian national standards for soft drink products[31] Fig.5.

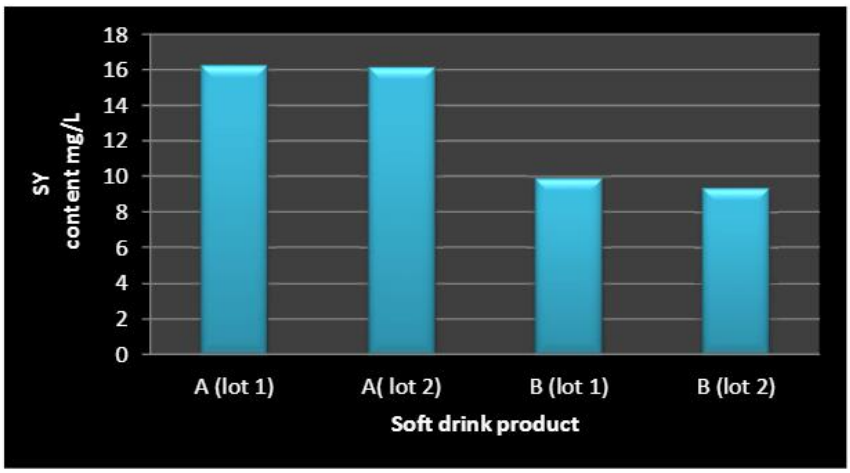


**Figure 4 SY contents in the studied samples of Fizzy drink using Column chromatography**

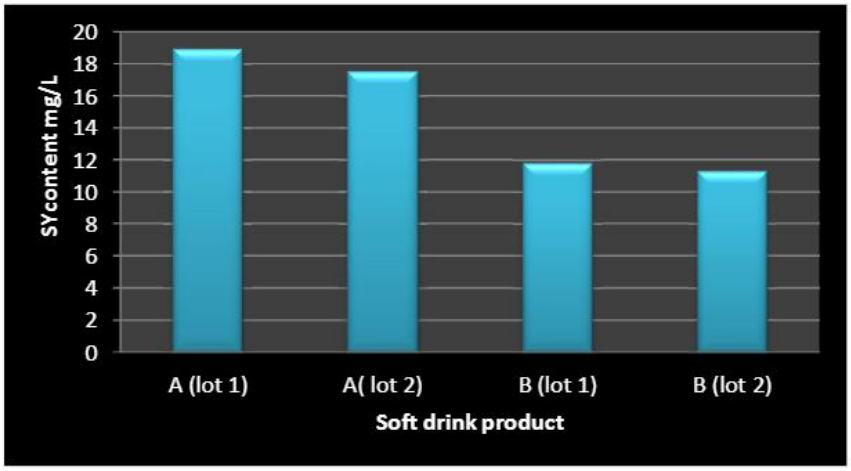


**Figure 5 SY contents in the studied samples of Fizzy drink using Wool dyeing**

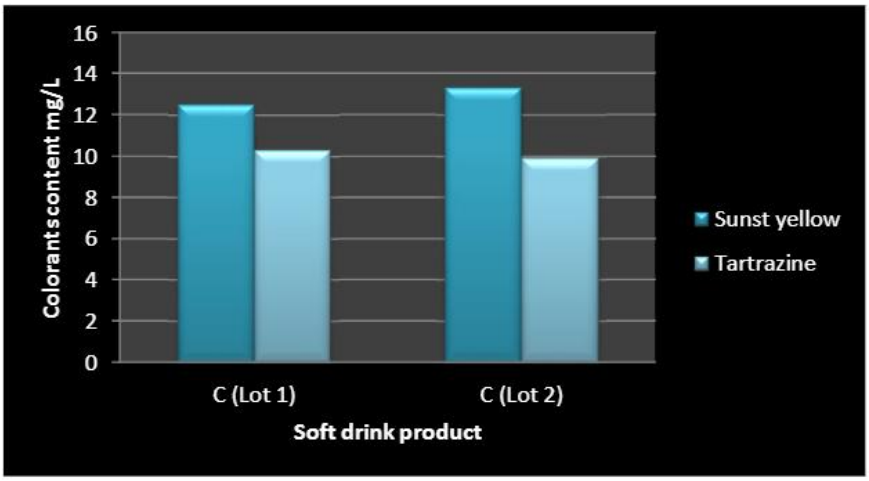


**Figure 6 SY and TAR contents in the studied samples of Fizzy drink using TLC**

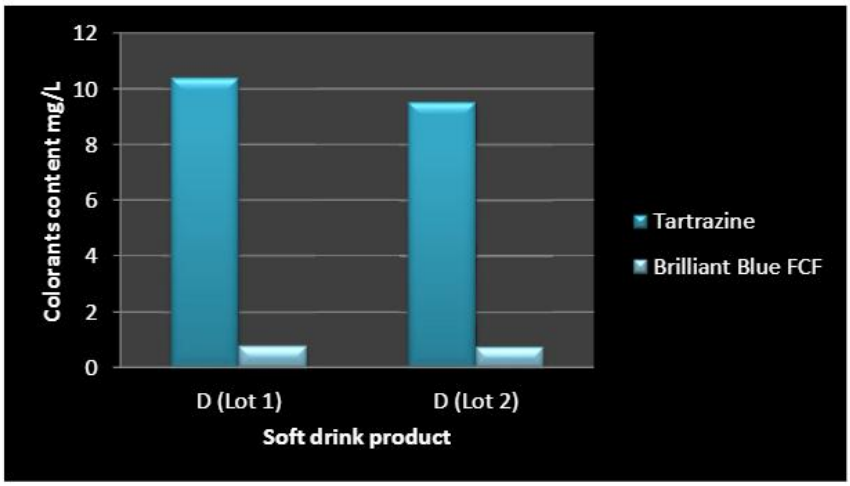


**Figure 7: SY and BB contents in the studied samples of Fizzy drink using TLC**

## 4. CONCLUSION

In this work, the levels of three common synthetic dyes of Tartrazine (T) Sunset Yellow (SY) and Brilliant Blue (BB) were detected and evaluated in commercial fizzy drinks of different qualities by different analytical methods. Two techniques of Wool dyeing and HPLC combined with UV detector were applied to determine SY as an individual additive dye. However, it was found that column chromatography technique was more precise than wool dyeing method with recoveries at 92% and 93% respectively. TLC separation and determination of binary mixtures of the studied colorants revealed precise findings with recovery more than 90%. In general, all studied samples of fizzy drink can be considered safe for consumption because they were contained permitted synthetic dyes at concentration lower than the max levels which were set in Syrian national standards for soft drink products.

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