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Chromatography Method for Simultaneous Determination of Five Synthetic Dyes by High Performance Liquid Chromatography

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Abstract
An effective analytical method for the simultaneous determination of five synthetic colorants (i.e., Tartrazine, Brilliant Black, Sunset yellow, Brilliant Blue and Erythrosine) in wine by Reversed-Phase High-Performance Liquid Chromatography with a Diode Array detector was established. The experimental parameters, including the spectrophotometric properties, detection limits and recoveries of studied synthetic colorants, were studied in detail. These parameters were determinated according to statistical methods.Under optimized conditions, the recoveries for all experimental samples were in the range of 98.03–103.5%, and this method had good linearities in the tested ranges with correlation coefficients (r2) >0.9991. The limits of quantification for five synthetic colorants were between 0.054 and 0.1 $mg.L^{-1}$.
Keywords: synthetic dyes, high performance liquid chromatography

1. Introduction

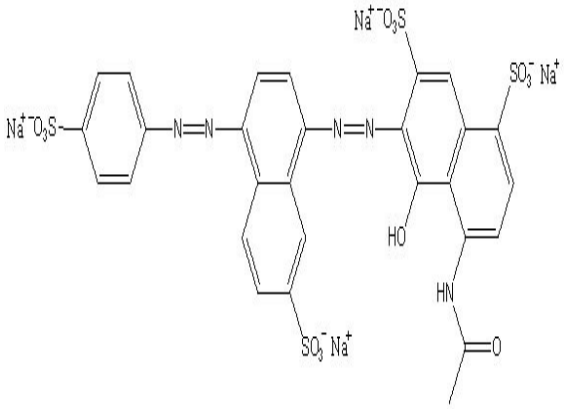
Food dyes are used for maintenance and improvement of color appearance in foods. In recent years, natural food dyes have been increasingly used for the consumer preference. However, they are relatively unstable and their costs are higher than synthetic food dyes. Therefore, synthetic food dyes are still used instead of natural dyes in many foods such as drinks, candies, and sweets.

Natural or synthetic food colorants are often added to foodstuffs and soft drinks in order to maintain the natural color during process or storage and to create the desired colored appearance. However, synthetic dyes have more advantages than natural dyes such as low price and high stability. At present, synthetic dye is widely used to make food more attractive and appetizing. Due to its toxicity, especially when consumed in excess, synthetic dyes is strictly controlled by laws, regulations and acceptable daily intake (ADI) values for food safety (Table 1).

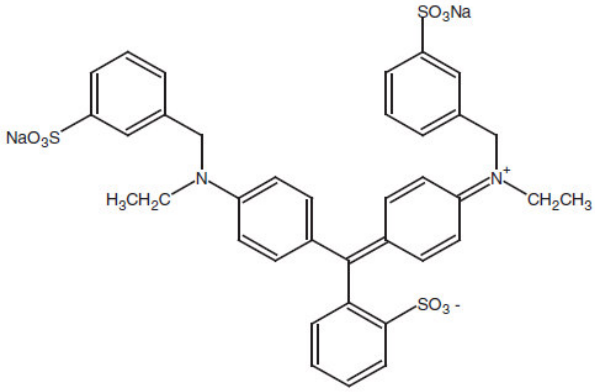
Table 1. Amount of synthetic dyes permitted by Ministry of Public Health (Thailand), Issue 281 (A.D.2547), acceptable daily intake (ADI) values for food safety and countries prohibiting of some of these dyes

Synthetic dye	Color	Maximum allowance limit (mg/Kg of food)	ADI (mg/Kg of body weight)	Use prohibited
Brilliant blue FCF	Blue	50	0-12.5	Belgium, France, Germany, Switzerland, Sweden, Austria, Norway
Tartrazine	Yellow	200	0-7.5	Norway, Austria
Sunset yellow FCF	Yellow	200	0-25	Norway

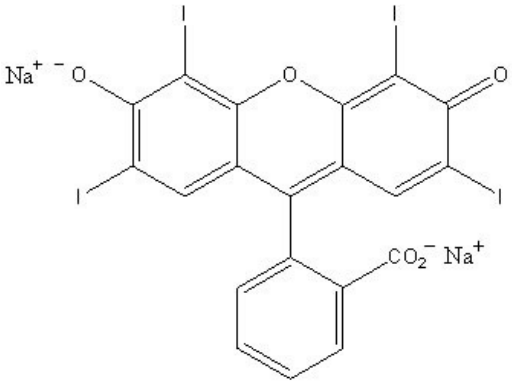
The chromophore groups in synthetic dyes (Figure1) can be analyzed with several methods such as visible spectrophotometry(1), thin layer chromatography(2), high performance liquid chromatography (HPLC)(3-4-9-10), capillary electrophoresis (CE)(5-6) and ion chromatography (IC)(7). Among the methods mentioned above, HPLC provided the highest sensitivity and the separation of synthetic dyes were performed on a reversed phase C18 column(3-4). While most HPLC and CE studies have showed the separation of red or the mixture of red and yellow synthetic dyes, only a few papers(7,8) have showed the separation of blue, red and yellow of synthetic dyes which were useful for the determination of dyes in real samples. Therefore, the purpose of this study was to develop HPLC method on RP for the separation of synthetic dyes over the range of blue, violet, yellow and red dyes within a relatively short analysis time and under high sensitivity. The representative dyes for this study were Brilliant Black BN, Brilliant Blue FCF, Erythrosine, Sunset yellow FCF and Tartrazine.



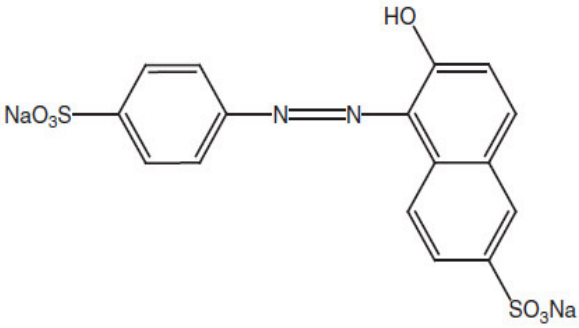
Brilliant black BN (E-151)



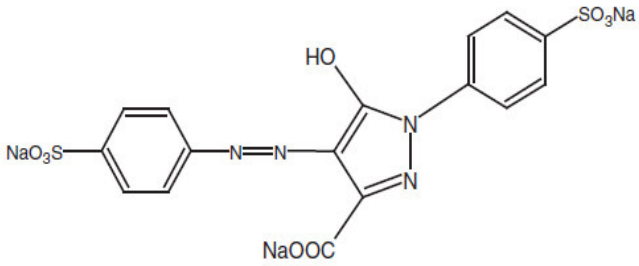
Brilliant blue FCF (E133)



Erythrosine (E-127)



Sunset yellow F.C.F (E-110)



Tartrazine (E-102)

Figure 1. Structure formula of synthetic dyes.

2. Materials and Apparatus:

2.1. Apparatus

The analyses were performed with a Shimadzu (Kyoto, Japan) LC-20 system equipped with LC-20AT pumps, CTO-20A/20AC column oven, SIL-20A/20AC auto sampler and a diode array detector SPD-M20A. Separations were done using a knaure C18 column (25cm x 4.6mm, 5µm). A Digital Ultrasonic cleaner (PHYLO) was used to degas the sample solution

2.2. Reagents and Solutions

The standard synthetic dyes were Brilliant black BN (E-151) from Aldrich Chem, Brilliant blue FCF (E133) from Loba Chemie, Erythrosine (E-127) from Loba Chemie, Sunset yellow F.C.F (E-110) from Tanya Exports and Tartrazine (E-102) from BDH Laboraty supplies. Stock solutions of synthetic dyes were prepared at a concentration of 1000 mg/L. All dyes were dissolved in deionized water. HPLC acetonitrile was obtained from Merck, methanol was from Sigma-Aldrich and potassium dihydrogen orthophosphate was from Labo Chemie.

3. Results and discussion

3.1. Absorption Spectra of Synthetic Dyes

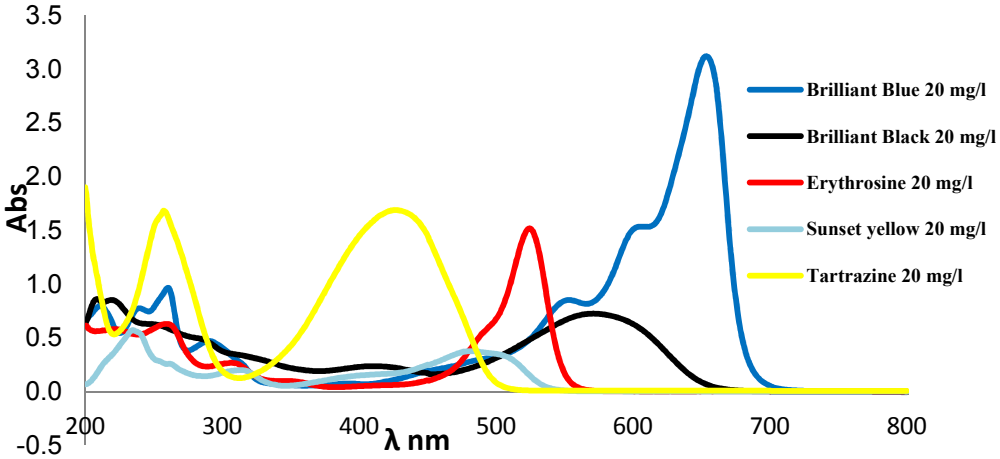
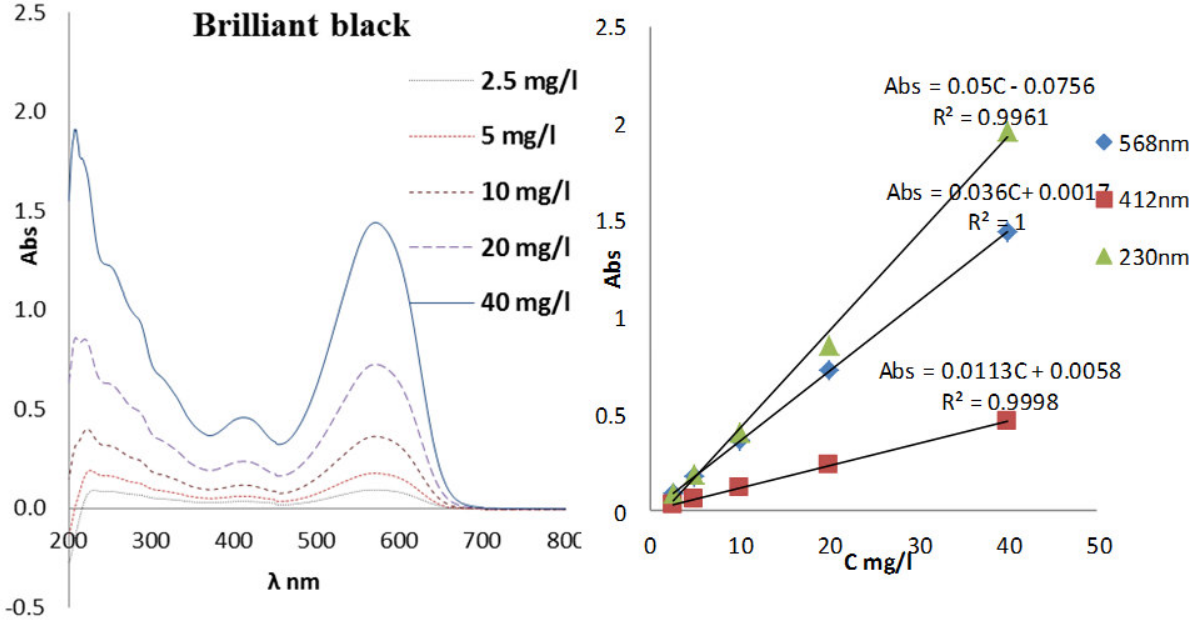


Figure 2. Absorption spectra of five synthetic dyes dissolved in water at pH 7

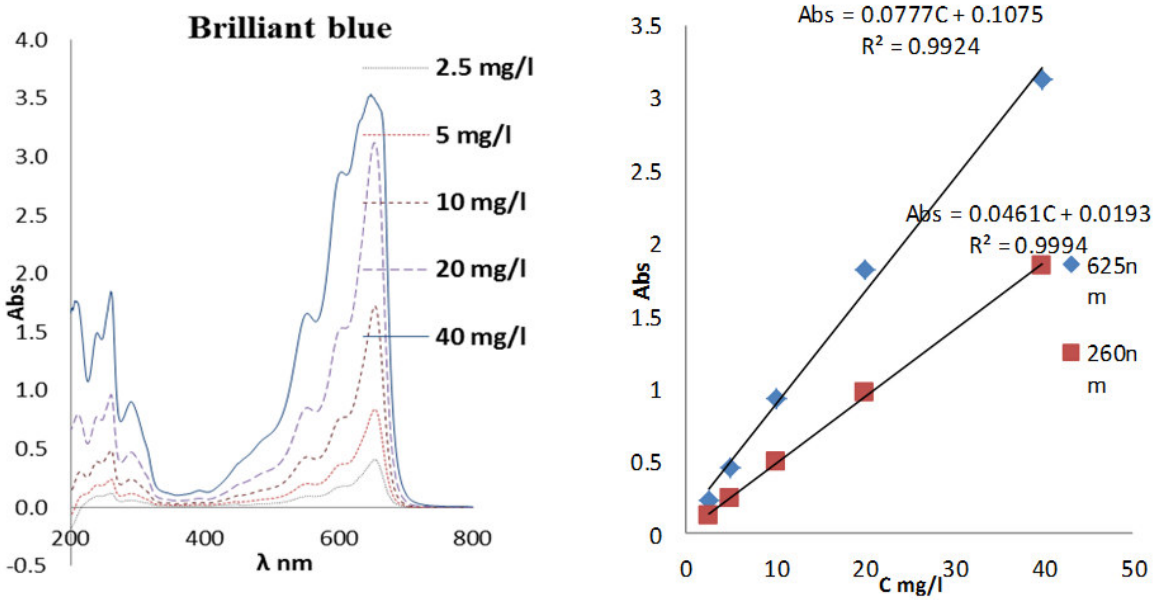
The Absorption spectra of each standard synthetic dye dissolved in water and measured at pH 7 are shown in figure 2. The maximum absorbance of each dye obtained in both UV and visible wavelength.

The spectrophotometric behavior for five water soluble synthetic dyes have been studied at the total UV-Vis range. First of all, the (λmax) for these synthetic dyes have been established: Brilliant black BN (E-151) (λmax= 230, 412, 568nm); Brilliant blue FCF (E133) (λmax= 260, 625nm); Erythrosine (E-127) (λmax= 260, 524nm); Sunset yellow F.C.F (E-110) (λmax= 240, 319, 481nm); Tartrazine (E-102) (λmax= 275, 425nm). Secondly, the linear relation between the absorbance and synthetic dye concentration has been determined. Figure (3-7)



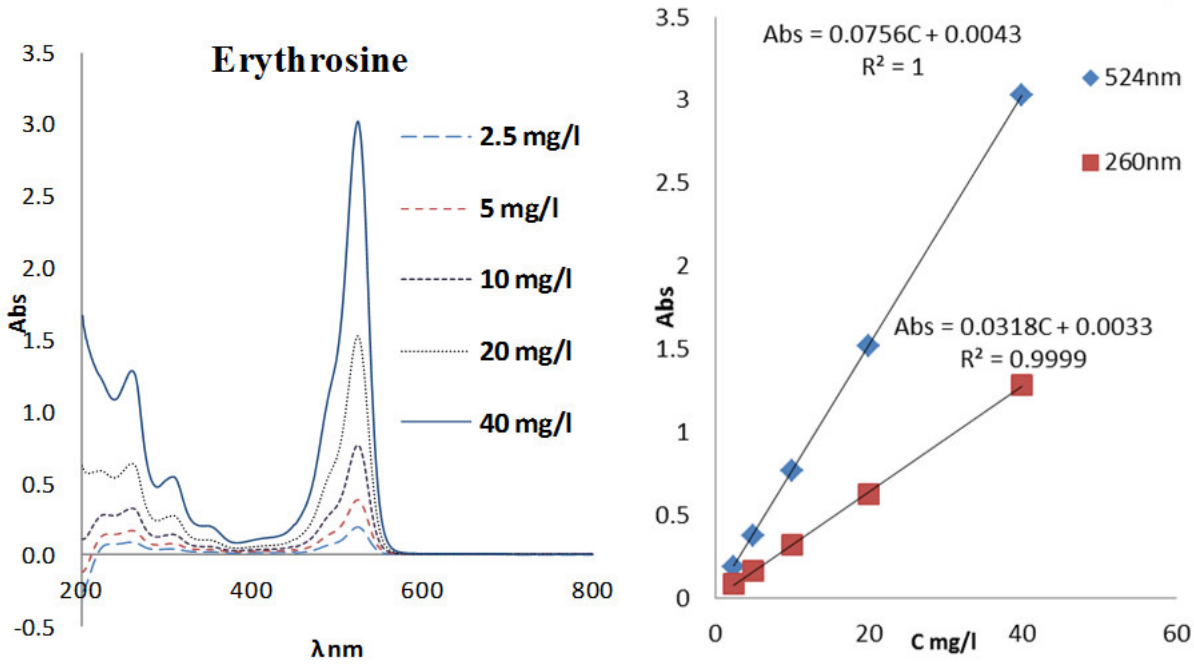
(a) (b)

Figure 3: a- The Molecular absorption spectra for different concentrations of Brilliant black NB
b- The linear relation between the absorbance and Brilliant black NB concentration



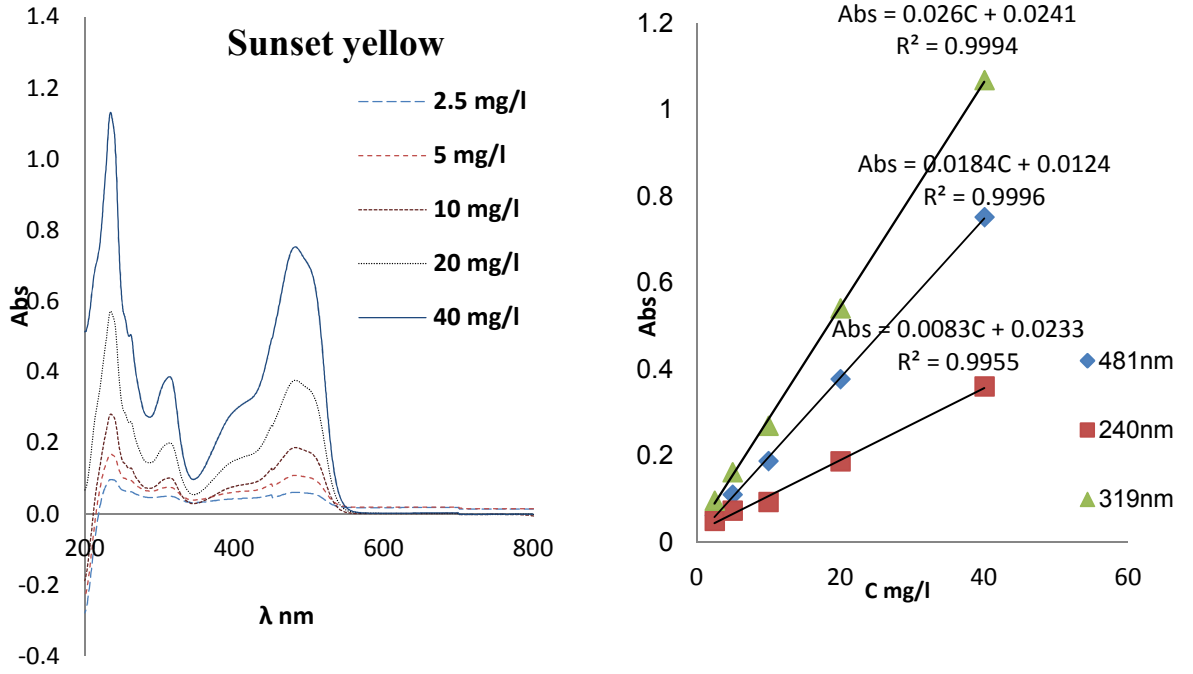
(a) (b)

Figure 4: a- The Molecular absorption spectra for different concentrations of Brilliant blue FCF
b- The linear relation between the absorbance and Brilliant blue FCF concentration



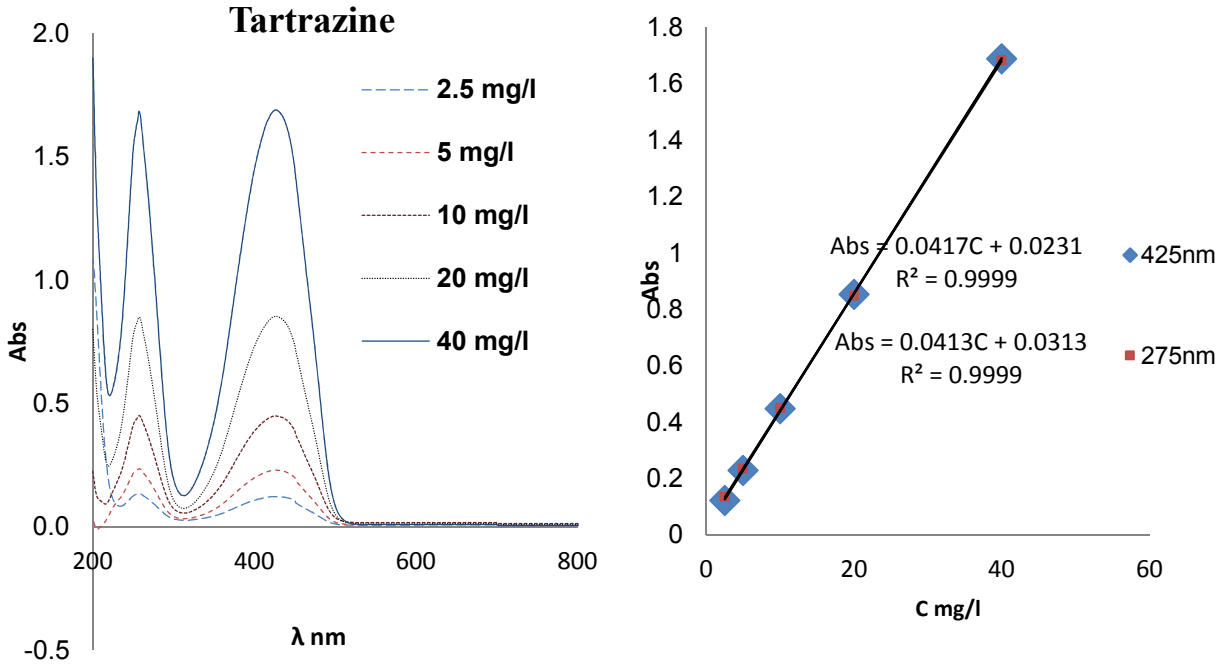
(a) **(b)**

Figure 5: a- The Molecular absorption spectra for different concentrations of Erythrosine
b- The linear relation between the absorbance and Erythrosine concentration



(a) (b)

Figure 6: a- The Molecular absorption spectra for different concentrations of Sunset yellow
b- The linear relation between the absorbance and Sunset yellow concentration



(a) (b)

Figure 7: a- The Molecular absorption spectra for different concentrations of Tartrazine
b- The linear relation between the absorbance and Tartrazine concentration

3.2. Liquid chromatography and separation conditions:

The mobile phase consisted of Solvent A which was [aqueous solution of 1.5mmol/l of potassium dihydrogen orthophosphates (pH 4.5)] and solvent B which was [methanol–acetonitrile (30:70, v/v)]. In gradient-elution analysis, like what it's shown in Table 2, The flow rate of the mobile phase was 1mL/min, the column temperature was kept at 40°C and the injection volume was 20µl. Detection wavelength for HPLC was selected in 254 nm.

Each analysis was performed in three replicates.
HPLC chromatograms of mixed food color standard solutions are shown in Figure 8.

Table 2. gradient of mobile phase with a time

Time min	0.11	3.50	11.30	12.50	15	17.50	20	22.10
A%	95	75	60	30	20	75	90	93
B%	5	25	40	70	80	25	10	7

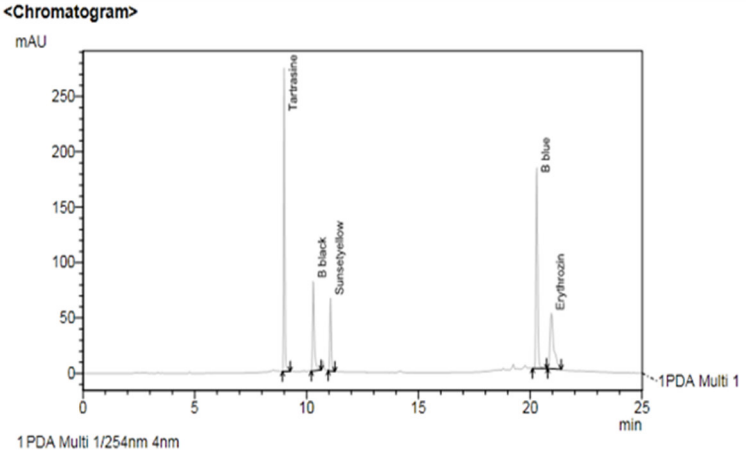


Figure 8. Figure 8. chromatogram of synthetic dyes (Tartrazine, Brilliant Black, Sunset yellow, Brilliant Blue, Erythrosine) standard solution (20 mg/l) mobile phase of (Solvent A* & Solvent B** In gradient-elution analysis) λ:254nm; F=1 mL/min; t=40 ºC *1.5mmol/l of potassium dihydrogen orthophosphates aqueous solution (pH 4.5) **methanol–acetonitrile (30:70, v/v).

3.3. Quantitation:

Each food dye has different wavelength of maximum absorption. We chose typical wavelength 254 nm.

The linearity for each compound was checked by analyzing standard solutions of different concentrations. The Calibration curve for Brilliant black BN standard solutions was linear ($r2$ = 0.9995) between 0.1mg/l and 20mg/l, for Brilliant blue FCF standard solutions was linear (r2 = 0.9993) between 0.5mg/l and 35mg/l, for Erythrosine standard solutions was linear (r2 = 0.9991) between 0.5mg/l and 30mg/l, for Sunset yellow FCF standard solutions was linear (r2 = 0.9996) between 0.1mg/l and 30mg/l, and for Tartrazine standard solutions was linear (r2 = 0.9997) between 0.1mg/l and 20mg/l.

The limit of quantitive (LOQ) for Brilliant black BN was 0.1 mg/l, for Brilliant blue FCF was 0.076 mg/l, for Erythrosine was 0.098mg/l, for Sunset yellow FCF was 0.054mg/l, for Tartrazine was 0.073 mg/l.

3.4. Recoveries:

Statistical treatments of the experimental results indicate that the proposed method is simple and sensitive . The precision and accuracy of the method were tested by analyzing three replicates of the synthetic dye. The low values of relative standard deviation (RSD%) indicate good precision and reproducibility of the method. Table 3.

Table 3. shows the recoveries of five food dyes from four experimental sample;

*(n = 3, α= 0.95,)(SD/$\sqrt{n}$× *δX =$t_{\alpha,k}$

Sample	C, mg/L		SD, mg/L	RSD%	X±δX *	R%
	Taken	Found				
Tartrazine	20	20.4161	0.0209	0.1023	0.051±20.4161	102.0805
	15	15.2475	0.0223	0.1462	0.0553±15.2475	101.65
	10	9.9916	0.0805	0.8056	0.1998±10.0365	99.916
	5	5.0584	0.0018	0.0361	0.0044±5.0584	101.168
Brilliant black	20	19.6315	0.0071	0.2722	0.0176±19.6315	98.1575
	15	15.1366	0.0177	0.1169	0.0439±15.1366	100.9106
	10	10.0381	0.0761	0.7525	0.1889±10.0381	100.381
	5	4.9540	0.0174	0.3512	0.0431±4.9540	99.08
Sunset yellow	20	20.4271	0.1318	0.6452	0.3267±20.4271	102.1355
	15	15.0280	0.0389	0.2588	0.0965±15.0280	100.1866
	10	10.1523	0.0937	0.9229	0.2326±10.1523	101.523
	5	4.9576	0.0057	0.1149	0.01415±4.9576	99.152
Brilliant blue	20	20.5152	0.0148	0.0721	0.0367±20.5152	102.576
	15	15.5309	0.0352	0.2266	0.0873±15.5309	103.5393
	10	10.0478	0.0194	0.193	0.0481±10.0478	100.478
	5	5.0540	0.0228	0.4511	0.0566±5.0450	101.08
Erythrosine	20	19.8563	0.0240	0.1208	0.0595±19.8563	99.2815
	15	14.9682	0.0234	0.1563	0.0580±14.9682	99.788
	10	9.8033	0.0732	0.7466	0.1817±9.8033	98.033
	5	5.0062	0.0232	0.4634	0.0575±5.0062	100.124

4. Conclusions

An analytical method was developed for quantitation of 5 synthetic food dyes by HPLC with diode array detection. Using a C18 analytical column, these food dyes were analyzed within 22 min. This method gave reliable and reproducible results with satisfactory detection limits and short analysis time for the routine analysis of food dyes.

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