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Original Article

SIMULTANEOUS DETERMINATION OF TARTRAZINE AND BRILLIANT BLUE IN FOODSTUFFS BY SPECTROPHOTOMETRIC METHOD

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ABSTRACT

Objective: Objective of the study was to develop a simple spectrophotometric method for simultaneous determination of two colorants, Tartrazine (T) and Brilliant Blue (BB), in foodstuffs without any prior treatment.

Methods: Derivative spectrophotometric (DS) and spectrophotometric methods were applied for the determination of (T) and (BB), respectively.

Results: Tartrazine was determined by first derivative spectrophotometry at 454 nm ($^1D_{454}$); where Brilliant blue was determined by zero-order spectrum at 630 nm. Linearity ranges were 2-24 5g/ml for (T) and 2-14 5g/ml for (BB), regression analysis showed a good correlation co-efficient R^2=0.9999 and R^2=0.9998 for (T) and (BB), respectively. The limit of detection (LOD) and limit of quantification (LOQ) was to be 0.12 and 0.35 5g/ml for (T), 0.17 and 0.52 5g/ml for (BB), respectively.

Conclusion: The proposed methods were successfully applied to analysis individual or mixture of Tartrazine and Brilliant Blue in foodstuffs**.** All studied samples showed dye levels conformity with Syrian legislation.

Keywords: Tartrazine, Brilliant Blue, Derivative Spectrophotometry.

INTRODUCTION

Colorants are the first sensory parameter by which food quality, making food more attractive. Synthetic colorants are usually added to food to replace the natural colorants that can be lost during processing or to avoid variations in the color of the final product. The trouble is that some synthetic Azo dyes such Tartrazine can be toxic to the human health when contact with some drugs can cause allergic to some people [1]. Also researches have shown that it can be linked to asthma, hyperactivity particularly among children [2].

Thus, various methods have been proposed to control the amount of colorants in food such as capillary zone electrophoresis (CZE)[3], differential pulse polarography [4-5] and voltametric methods as adsorptive voltametery [6]. Chromatographic methods have been used for colorants analysis in foods, high performance liquid chromatography (HPLC) [7], high-performance ion chromatography [8], reversed-phase thin-layer plates [9], reverse phase high performance liquid chromatographic (RP-HPLC) method [10, 11] and micellar electrokinetic capillary chromatography (MEKC) [12].

Spectrophotometric [13, 14] and derivative Spectrophotometric methods [15, 16] are successfully applied to determine synthetic dyes.

The aim of this work is to develop the simple and accurate spectrophotometric method for simultaneous determination of BB (E133) fig.1, T (E102) fig. 2 and their mixtures in different foodstuffs without prior treatment by UV/Vis spectrophotometry and derivative spectrophotometry (DS), so that their mixtures give green shades in products.

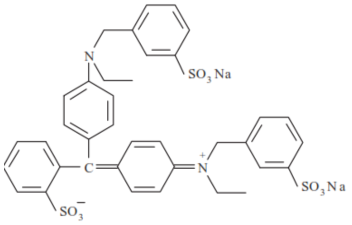

Fig. 1: Structural formula of Brilliant Blue

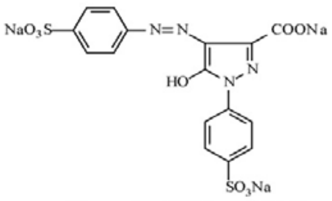

Fig. 2: Structural formula of Tartrazine

MATERIALS AND METHODS

All spectral measurements were carried out using a T80+UV/V spectrophotometer connected to computer, quartz cells 1 cm. Diahan ultrasonic bath and Centurion Scientific Ltd centrifuge. Distilled water was used to prepare the solutions. The standard synthetic colorants were Brilliant Blue (CI 42090, CAS 3844-45-9) purity 95% and Tartrazine (CI 19140, CAS 1934-21-0) purity 94.5%. Both colorants were obtained from Sineset (French).

Samples

Several trademarks samples were purchased from Syrian local markets, including (Kinan and yellow chewing-gum, Mirinda soft drink, Windmill powder juice, Diva and Samiry candies).

Solutions

Stock solutions (100 5g/ml) of Tartrazine and Brilliant Blue were prepared by dissolving appropriate weights of pure colorants in distilled water.

The working standard solutions of each colorant were prepared by appropriate dilutions of stock solutions with distilled water to give concentrations between 1.2-30 5g/ml of (T) and 1.2-20 5g/ml of (BB), taking the purity of the colorants on consideration.

Samples preparation

Seven products were studied

- Twenty Diva candies pieces (green apple) were weighed and crushed. An accurately weighed sample equivalent to nine pieces was dissolved in distilled water, and then the solution was transferred into 50 mL volumetric flask.

- Yellow Diva candies lemon flavour prepared at the same past way by weighing ten pieces, then weighed sample equivalent to five pieces, then solution transferred into 25 mL volumetric flask.
- Twelve Kinan chewing gums were taken and extracted colorant by dissolving it in distilled water until removing colorant from gums, then the solution was transferred into 25 mL volumetric flask and centrifuged during 15 min at 5000 rpm.
- Five pieces of yellow gums were taken and completed the colorant extraction as a Kinan chewing gum procedure.
- Mirinda (green apple) soft drink sample was previously degassed in an ultrasonic bath for 15 min. An aliquot sample was analyzed without any dilution.
- Windmill powder juice lemon flavour sample was homogenized. 1 g was accurately weighted and dissolved in distilled water, then the solution was transferred into a 25 ml volumetric flask. The sample solution was centrifuged during 15 min at 5000 rpm.
- Suitable pieces number of blue Samiry candies sample was taken and completed the colorant extraction as a chewing-gum procedure.

RESULTS AND DISCUSSION

Absorption spectra of the standard colorants 6 µg/ml (T) and 6 µg/ml (BB) solutions were recorded within a wavelength range of 300–700 nm against distilled water fig. 3. As can be seen, (BB) can be determined by direct measurement of absorbance at 630 nm, but (T) cannot because of the overlapped spectra.

On the other hand, derivative spectrophotometry showed more resolution, where it made the determination of (T) and (BB) mixture possible without pretreatment.

The first derivative spectra at zero-crossing point was used to determine (T) in the presence of (BB) at 454 nm (fig. 4).

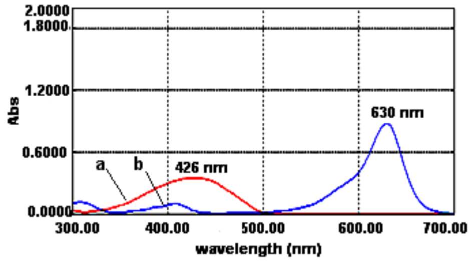

Fig. 3: Zero-order spectra of: a-(T), b–(BB)

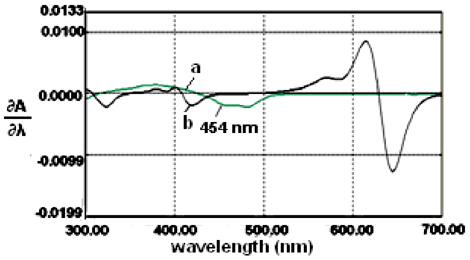

Fig. 4: First derivative spectra of: a-(T), b–(BB)

Methods validation

The validity of the proposed methods was assessed by accuracy (reported as the percentage recovered), precision (reported as RSD %), linearity (evaluated by regression equation), limit of detection (LOD) and limit of quantification (LOQ).

Linearity

The concentration linearity of (T) was to be in the range 2-24 5g/ml at 454 nm by $^{1}D_{454}$ fig. 5 & 6 and the concentration linearity of (BB) was to be in the range 2-14 µg/ml by zero-order absorption spectra at 630 nm fig. 7 & 8.

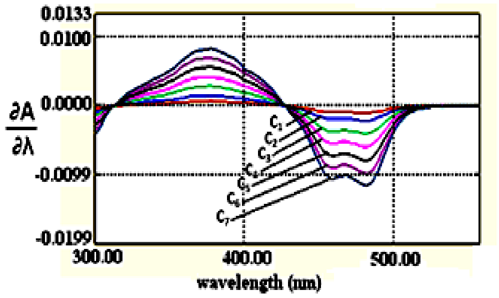

Fig. 5: First derivative spectra of (T):
C_1: 2 µg/ml, C_2: 4 µg/ml, C_3: 8 µg/ml, C_4:12 µg/ml, C_5: 16 µg/ml, C_6: 20 µg/ml C_7:24 µg/ml.

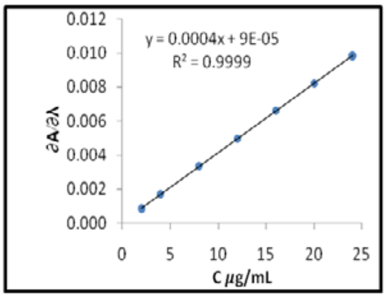

Fig. 6: Calibration curve for (T)
n=5 for each concentration.

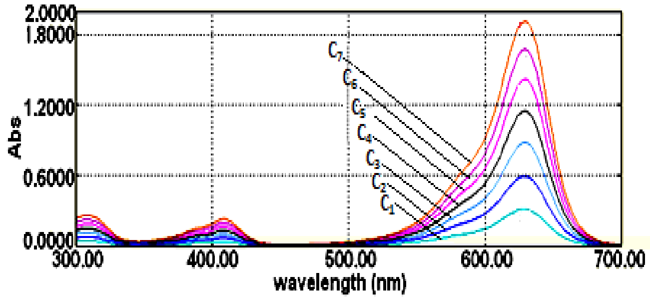

Fig. 7: Zero-order spectra of (BB):
C_1: 2 µg/ml, C_2: 4 µg/ml, C_3: 6 µg/ml,
C_4: 8 µg/ml, C_5:10 µg/ml, C_6:12 µg/ml,
C_7:14 µg/ml.

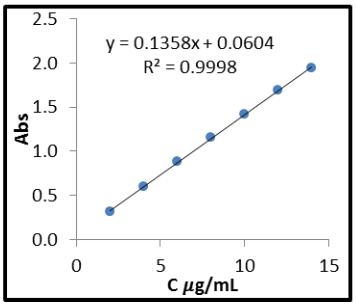

Fig. 8: Calibration curve for (BB)
n=5 for each concentration.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated using the following equations:

$$\mathrm{LOD} = \frac{3.3 \times \mathrm{SD}}{\mathrm{m}} ; \quad \mathrm{LOQ} = \frac{10 \times \mathrm{SD}}{\mathrm{m}}$$

Where SD is the standard deviation of y-intercepts of regression lines and m is the slope of the calibration curve.

Accuracy and precision

To determine the precision and accuracy of the proposed methods, five replicate determinations were carried out on three different concentrations of standards (BB) and (T). The validation results are shown in tables 1 and 2.

Analytical application

The developed method was applied for quantitative determination for food colorants in different foodstuffs from Syrian local markets. The samples were prepared as described in the section of samples preparation and analyzed. Quantitative analysis were done by using calibration curves and addition standard curves. The obtained results are summarized in table 3, at ten different samples for each product.

In general, the used amounts of (T) were relatively higher than those of (BB) in all analyzed products. However, the concentrations of the detected colorants were much lower than the Syrian limits [17]: 500 mg/kg for (T) and 200 mg/kg for (BB).

The relative standard deviations RSD % (n=5) of the quantitative results were in the range of 0.50-3.48 % and 0.15-2.77 % for (T) and (BB), respectively.

Table 1: Statistical data for calibration graphs

Method	Analyte	Selected wavelength (nm)	Linearity rang μg/ml	Correlation coef. (R^2)	LOD μg/ml	LOQ μg/ml
DS	T	$^{1}D_{454}$	2-24	0.9999	0.12	0.35
Zero-order spectrum	BB	$\lambda_{max,\ 630}$	2-14	0.9998	0.17	0.52

Table 2: Method validation for the simultaneous determination of Brilliant Blue and Tatrazine by the proposed methods

Method	Colorant	theoretical concentration (µg/ml)	*observed concentration (µg/ml)	Precision RSD(%)	Accuracy (%)
Zero-order spectrum	Brilliant Blue	4	3.99	1.80	99.75
		8	8.08	0.23	101.00
		12	11.99	0.47	99.91
DS	Tatrazine	4	4.07	2.45	101.75
		8	8.22	1.33	102.75
		12	12.22	0.90	101.83

Accuracy (%) = (observed concentration/theoretical concentration) × 100. Precision (%) = (standard deviation/mean concentration) ×100. * Five separate determinations were performed and mean calculated.

Table 3 (a-g): Results of analyzing Syrian trademarks of soft drink, chewing-gums, candies, and juice powder

Table a: Diva candy (Green Apple) trademark

No. of sample	Tartrazine		Brilliant blue	
	Concentration µg/piece	RSD %	Concentration* µg/piece	RSD %
1	2.27	3.05	0.41	1.36
2	2.41	2.88	0.43	1.98
3	1.78	3.46	0.44	1.44
4	2.48	2.80	0.47	0.68
5	2.55	2.72	0.41	0.79
6	2.75	2.52	0.48	0.68
7	2.29	3.32	0.41	1.33
8	2.35	2.64	0.46	0.69
9	2.49	2.49	0.49	0.65
10	2.63	2.36	0.49	0.66
Range	1.78-2.75		0.41-0.49	

*BB was determined by addition standard curve in Diva candy product.

Table b: Kinan gum (Green) trademark

No. of sample	Tartrazine		Brilliant blue	
	Concentration µg/piece	RSD %	Concentration µg/piece	RSD %
1	2.22	2.09	0.18	1.34
2	2.95	1.58	0.42	0.87
4	2.57	2.21	0.29	0.43
5	4.27	1.87	0.58	0.38
6	2.59	2.01	0.27	1.24
7	4.37	2.23	0.58	0.32
8	2.32	2.00	0.28	0.78
9	2.53	1.84	0.27	0.53
10	4.41	2.21	0.57	0.45
Range	2.22-4.41		0.18-0.58	

Table c: Mirinda (Green Apple) trademark

No. of sample	**Tartrazine**		**Brilliant Blue**	
	Concentration µg/ml	**RSD %**	**Concentration* µg/ml**	**RSD %**
1	9.48	1.17	0.51	2.30
2	10.13	1.35	0.50	2.00
3	9.73	1.14	0.59	1.95
4	10.58	1.05	0.54	2.77
5	10.33	1.08	0.51	2.13
6	9.33	1.19	0.50	1.17
7	10.23	1.09	0.52	2.22
8	10.18	2.19	0.53	1.08
9	9.38	2.38	0.57	1.78
10	9.83	1.13	0.56	1.04
Range	9.33-10.58		0.50-0.59	

* BB was determined by addition standard curve in Mirinda product.

Table d: Diva candy (Lemon) trademark

No. of sample	**Tartrazine**		**Brilliant Blue**	
	Concentration µg/piece	**RSD %**	**Concentration**	**RSD %**
1	2.23	1.22	-	-
2	2.96	0.75	-	-
3	1.99	2.80	-	-
4	3.06	0.91	-	-
5	1.91	2.91	-	-
6	1.89	2.41	-	-
7	2.40	1.46	-	-
8	1.87	1.17	-	-
9	2.09	1.06	-	-
10	1.96	3.48	-	-
Range	1.87-3.06			

Table e: Samiry candy trademark

No. of sample	**Tartrazine**		**Brilliant Blue**	
	Concentration	**RSD %**	**Concentration µg/piece**	**RSD %**
1	-	-	0.32	1.98
2	-	-	1.09	0.18
3	-	-	1.47	0.71
4	-	-	0.87	0.39
5	-	-	0.34	0.49
6	-	-	2.28	0.66
7	-	-	2.50	0.46
8	-	-	1.97	1.20
9	-	-	0.79	0.15
10	-	-	0.64	1.04
Range		**e:** Samiry candy trademark.	0.32-2.50	

Table f: Yellow gum trademark

No. of sample	**Tartrazine**		**Brilliant Blue**	
	Concentration µg/piece	**RSD %**	**Concentration**	**RSD %**
1	5.08	1.92	-	-
2	6.73	1.64	-	-
3	7.58	1.47	-	-
4	4.42	0.50	-	-
5	7.23	1.54	-	-
6	6.98	1.60	-	-
7	6.43	2.12	-	-
8	7.33	1.53	-	-
9	6.04	2.31	-	-
10	7.10	2.41	-	-
Range	4.42-7.58			

Table g: Windmill powder juice (Lemon) trademark

No. of sample	**Tartrazine**		**Brilliant Blue**	
	Concentration μg/g	**RSD %**	**Concentration**	**RSD %**
1	10.73	1.04	-	-
2	10.43	1.31	-	-
3	11.48	0.97	-	-
4	11.98	0.93	-	-
5	11.73	0.95	-	-
6	12.23	0.91	-	-
7	12.67	1.08	-	-
8	11.33	0.98	-	-
9	12.73	0.87	-	-
10	12.68	1.07	-	-
Range	10.43-12.73		-	-

CONCLUSION

Tartrazine (T) and Brilliant Blue (BB) levels were estimated alone and in their mixtures in different studied local foodstuffs by simple spectrophotometric method. (BB) was determined directly by zero order spectra, but (T) was determined by the first derivative spectrophotometric method using zero-crossing point.

Colorants levels were very lower than their maximum values which established by the Syrian legislation. The proposed methods of (T) and (BB) determination are accurate, simple, sensitive, easy and directly applicable in quantitative analysis without previous chemical treatment in individual or binary mixture.

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CONFLICT OF INTERESTS

Declared None

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