

Determination of the Concentration of Food Azo Dyes by High Performance Liquid Chromatography (HPLC)

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ABSTRACT

Food additives are commonly used in processed foodstuffs to improve appearance, flavor, taste, color, texture, nutritive value and conservation. Thirty five different kinds of foodstuffs: solid juice powders, solid jelly powders and soft drinks, gum, fruit juice, candy, ice cream and soft jelly. High performance liquid chromatography (HPLC) involves direct injection of foodstuff homogenized, degassed and filtered with 0.20µm with visible detection at a single wavelength. UV detector (524 nm), column(C18, 250 × 4.6mm, id 5 µm) mobile phase acetonitrile: water: di hydrogen orthophosphate (40:40:20), flow rate 0.5 ml/min. Amaranth, fast green, Allura Red, Sunset Yellow, Tartrazine was used for determination of the concentration of five dyes, Sample preparation consisted of dissolving and filtering the samples before chromatographic test. Some products showed poor quality and/or production controls due to high concentration of dyes. The highest value obtained in gum, juice and ice cream products. The concentration of five dyes were ranged from 747.5 to 2255.25 µg/g for Allura Red, 5.86-991.93 µg/g for Sunset yellow, 513.67µg/ml - 370.704µg/mg for Amaranth, 370.704µg/gm-4.0µg/mg for Fast green, 24.65µg/ml-82.0µg/mg for Tartrazine. This fact was more critical for Sunset Yellow, fast green, in solid juice powders, colored gum, soft jelly there is concern that these substances can exceed legislated values. It was also observed that different producers use different dyes and/or composition in similar products.

Keywords: Determination, concentration, Azo dyes, HPLC technique, food additives.

1. INTRODUCTION

Dyes are a class of food additives without nutritional value which are added to foods with the objective of providing color thus making the product more attractive and increasing its consumer acceptability [1]. Amaranth, Allura red, Tartrazine, sunset yellow, Fast Green was classified as azo dyes with one or more azo bonds(-N=N-) [2,3]. It is approved for use in beverages, bakery goods, dessert powders, candies, cereals, drugs, and cosmetics. The Food and Drug Administration (FDA) determined the acceptable daily intake (ADI) of Tartrazine is 5 mg/kg body weight(bw)/day, Allura red is 7 mg/kg bw/day, Amaranth 0.8 mg/kg/bw/day, Sunset yellow 3.75 mg/kg bw/day, Fast green 2.5 mg/kg bw/day. Azo dyes and their metabolites are reported to be mutagenic or carcinogenic [4]. Azo dye compounds have been reported to cause bladder

cancer in humans and hepatocarcinoma, nuclear anomalies in intestinal epithelial cells in mouse models [5,6,7]. Many of the studies concluded that they cause and-effect relationship between food dyes and hyperactivity. The authors stated that dyes "promote hyperactivity in hyperactive children, as measured on behavioral rating scales" and that "society should engage in a broader discussion about whether the aesthetic and commercial rationale for the use of [artificial food colorings] is justified" [8] several cases of Tartrazine -sensitivity were reported, most frequently in the form of urticaria (hives) and asthma [9] genotoxicity studies performed on Allura Red 40 was positive in *in vivo* comet assay in the glandular stomach, lungs, and colon of mice [10] which indicates that Allura Red can cause DNA damage *in vivo*.

Depending on the acute toxicity of azo dyes, according to the criteria of the European Union for the classification of dangerous substances the values of LD50 are 250-2000 mg/Kg body weight [11]. The toxic effects was evaluated of two azo dyes used as food additives, Tartrazine and Carmoisine, by oral administration of two concentrations (one low and other high), in albino male rats, for 30 days. It was measured the quantities of urea, creatinine, total protein, albumin, lipid profile[12], blood glucose in serum, and estimated the activities of catalase in the hepatic tissue of the animals. . Many food dyes are controlled or even forbidden in many places of the world. Only Tartrazine (E-102), Sunset Yellow (E-110), Amaranth (E-123), Ponceau 4R (E-124), Red 40 (E-129), Eritrosine (E-127), Indigotine (E-132) and Brilliant Blue (E-133) are allowed in food [13].Data showed a significant increase in the rates of urea, creatinine, total protein and albumin in the serum of rats treated with Tartrazine and Carmoisine, especially in the higher concentrations. The activities of catalase decreased in the tissues of rats fed with the high dose of Tartrazine, high and low doses of Carmoisine it was concluded, therefore, that both azo dyes affected adversely and altered the biochemical markers of vital organs such as liver and kidney, not only in higher concentrations but also in the lowers. Tartrazine and Carmoisine not only cause changes in the hepatic and renal parameters but their effects become a risk to the organisms at higher doses, since it can induce oxidative stress by means of the formation of free radicals [14,15]. Large number of techniques have been employed for the analysis of azo dyes in foodstuff Thin Layer Chromatography [16] Capillary Electrophoresis [17, 18] reversed-phase liquid chromatography [19], Isocratic High-Performance Liquid Chromatography [20].

In Iraq, hyperactivity allergy was appeared in children in recent years because most of these foodstuff products contain one or more azo dyes, absent of health control and Iraqi market open to different products from neighbor countries and local manufactured products and these azo dyes were sold in local market. The aim of this study is to estimate the concentration of five azo dyes in children food samples.

2. MATERIALS AND METHODS

2.1 Samples

Samples consisted of solid jelly powder (pine apple, grape, strawberry and peach flavors), solid juice powder for drinks (orange, mango, cashew, pineapple, passion fruit and strawberry flavors), soft drinks (orange , grape, peach flavors),colored gum (red ,green, orange, yellow, pink),candies (strawberry, orange, mint, lemon flavors),flavored milk (strawberry, banana),soft jelly (red, yellow, orange, pink) . Thirty five of different samples were bought from super markets of Baghdad city, Iraq. Some of these samples were sufficient products without label and they were local products.

2.2 Sample preparation procedures

Solid samples were previously homogenized in their own packages before sampling. Sample of 5 g were precisely weighted. Solid juice powder samples were directly dissolved in ultra-pure water at room temperature. Solid jelly powder samples were dissolved in hot ultra-pure water (60° C). In both cases they were diluted up to 50 mL with ultra-pure water . Soft drink samples were previously degassed in ultrasonic bath,10 ml of each sample transferred to a 10ml of polypropylene centrifuge tube ,a 20 min centrifugation at 10000 rpm , filtered through 0.20 µm(chm) filters and directly analyzed [21].

2.3 Standard Calibration Curve

All standard solid dyes were purchased from sigma com. Of five dyes Allura Red(E129),Tartrazin (E102) , Amaranth (E123) , Sunset yellow (E 110) , Fast green (E143). The standard stock solution was diluted with ultra-pure water containing 1mg/ 10 ml of each dye in volumetric flask of 50 ml ,the volume made up to 50ml with ultra pure water. Optimum absorption wavelengths for each dye were previously evaluated using standard solutions. For quantitative analysis the following wavelengths were used Amaranth (524 nm),Allura Red (508 nm), Sunset Yellow (484 nm) and Tartrazin (454 nm). The characteristics of the UV detector [21].

2.4 HPLC Analysis

The HPLC system consisted of pump being Sykam model 1122 solvent delivery system, the injector was auto injector Sykam model S 5200,Oven was Sykam model S 4011 column thermo controller, detector was sykam model S 3240 UV multichannel detector, column was Supelco (250×4.6 mm, 5µm, ODS C18). The high performance liquid chromatography (HPLC) was used for analyzed all samples, Chromatography conditions (mobile phase composition and flow rate) were evaluated and optimized in column(C18, 250 × 4.6 mm, id 5µm), the UV detection was operated at (524 nm) for all azodyes, mobile phase acetonitrile: water: di hydrogen orthophosphate (40:40:20), flow rate 0.5 ml/min.

3. RESULTS AND DISCUSSION

This optimized mobile phase separated Tartrazin, Allura Red, Amaranth, Sunset yellow, Fast green at (4.25,10.49 ,6.91 , 8.08, 3.84 min.) retention time respectively. The calibration curve for all five dyes was obtained in Figure (1,2,3,4,5) for (Tartrazin, Allura Red, Amaranth, Fast green Sunset yellow) respectively. Optimum absorption wavelengths for each dye were previously evaluated using standard solutions. A survey determination of five dyes in food products manufactured in Iraq and imported products. The results are summarized in 2225.25µg/gm for Allura Red to 4.0 µg/gm for Tartrazin products manufactured food Using the UV detector, specific spectra could be confirmed from the peaks of in all products was below the limit of detection in commercial products, which implies that the products manufactured contained

marginal amounts. Standard calibration curve was summarized in fig.(1,2,3,4,5) for Tartrazin, Allura Red, Amaranth, Fast green, Sunset yellow respectively.

Samples detection limits determined in fig. (6,7,8,9,10) with pure peaks in some samples.

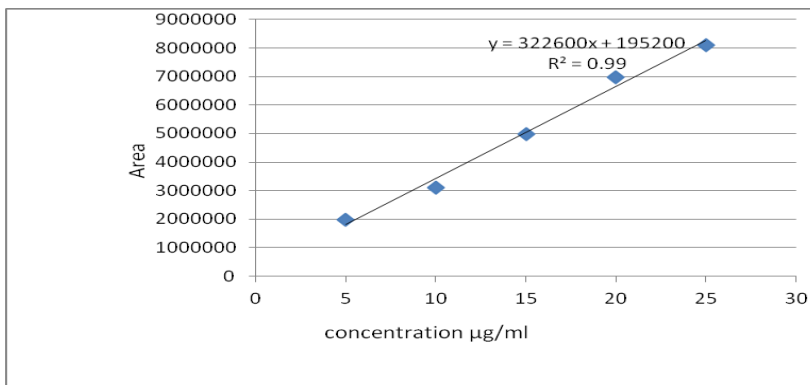


Figure 1. Standard curve of Allura red

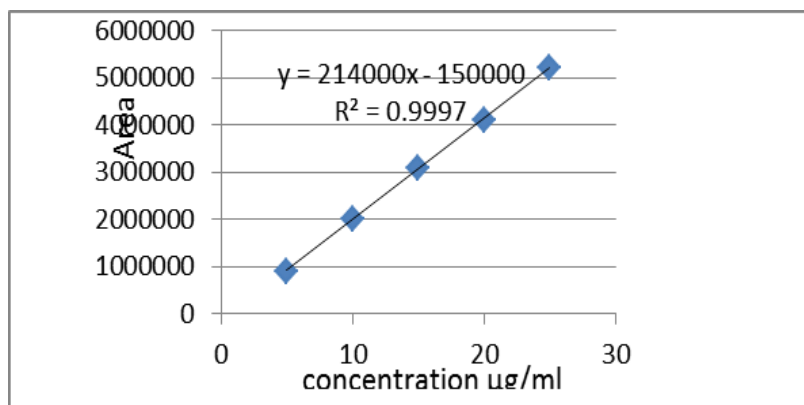


Figure 2. Standard curve of Amaranth

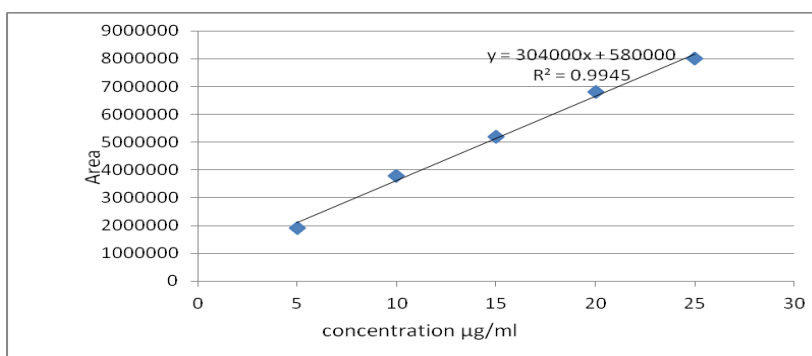


Figure 3. Standard curve of sunset yellow

Table (1) summarized the concentration of all five dyes in different types of food products, Allura red, Amaranth, sunset yellow and fast green was the highest value in gum, juice and ice cream products. Thirteen products appeared pure peaks with one type of azodyes in different wave length which indicates that the

products contained different types of azodyes or additives, twenty products appeared with many different peaks and in different concentrations. Even the low concentration of Tartrazin but its high value comparable to the international acceptable value.

Table 1. Concentration of azodyes in different samples.

No.	Samples	Azo dyes	Concentration * $\mu\text{g/g}$,** $\mu\text{g/ml}$
1	soft drink	Tartarazin	24.65
2	Juice powder	Tartrazin	82.
3	Fruit juice	Allura red	2255.25
4	jelly powder	Allura red	898.45
5	Gum(red)	Allura red	747.5
6	Fruit juice	Amaranth	513.67
7	Gum(pink)	Amaranth	370.704
8	ice cream	Green	384.91
9	Gum (green)	Green 5	209.34
10	M&Ms	Green	4
11	Gum (orange)	Sunset yellow	991.93
12	soft drink	Sunset yellow	425.47
13	M &MS	Sunset yellow	5.86

* $\mu\text{g/gm}$:for solid
** $\mu\text{g/ml}$:for liquid

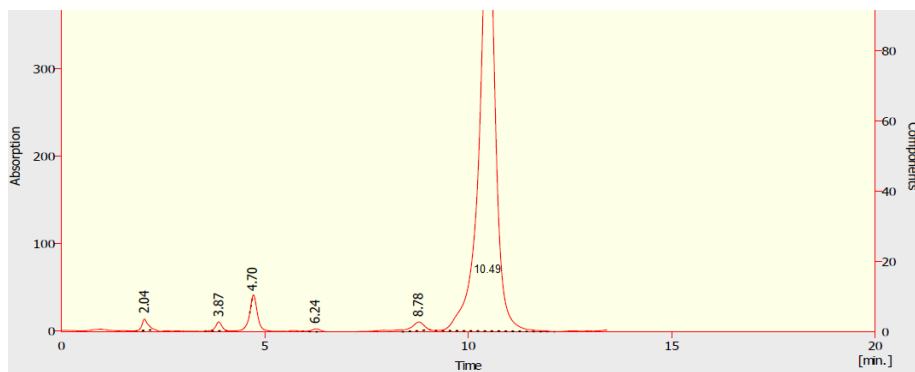
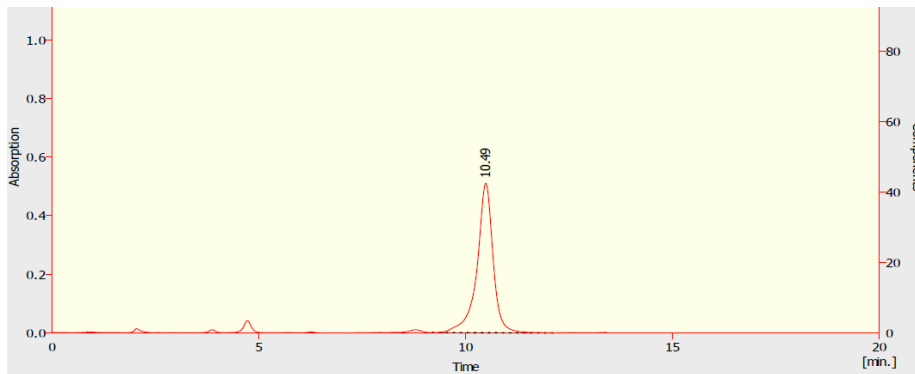
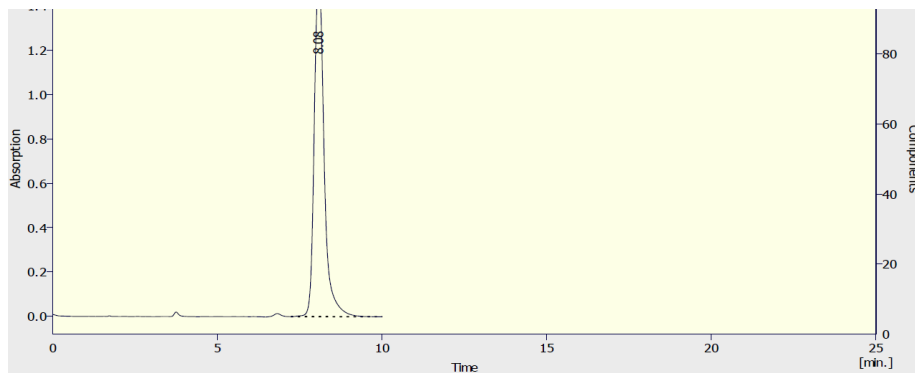


Figure 4. (A) chromatography of Allura red standard (B) chromatography of Allura red in Fruit juice



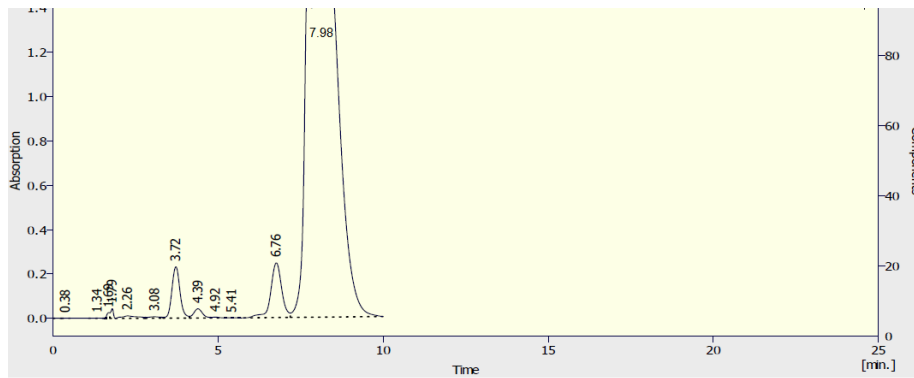


Figure 5. (A) chromatography of sunset yellow standard (B) chromatography of sunset yellow in gum.

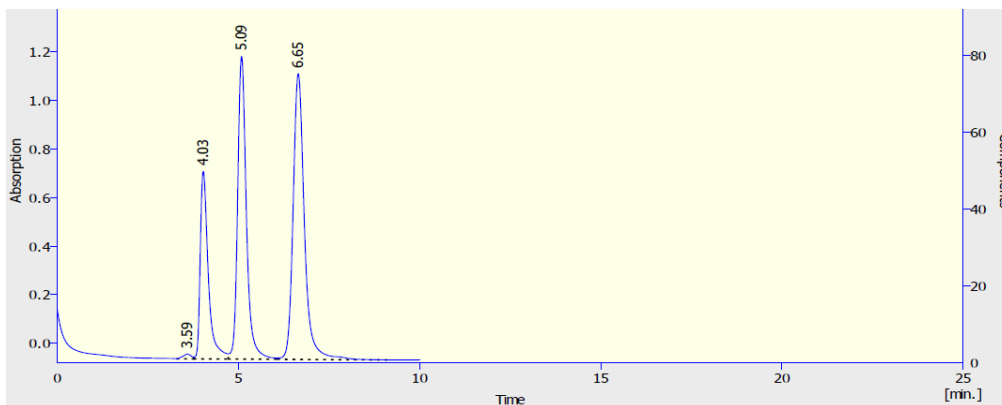
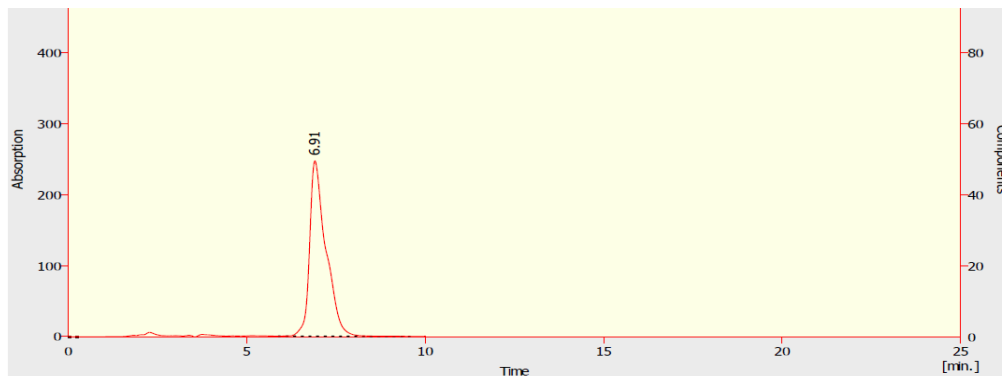


Figure 6.(A) chromatography of Amaranth standard (B) chromatography of Fruit juice.

The concentration of five dyes were ranged from 747.5 to 2255.25 $\mu\text{g/g}$. for (Allura red) , 5.86-991.93 $\mu\text{g/g}$, for sunset yellow, 513.67 $\mu\text{g/ml}$ - 370.704 $\mu\text{g/mg}$ for Amaranth, 370.704 $\mu\text{g/g}$ -4.0 $\mu\text{g/mg}$ for fast green, 24.65 $\mu\text{g/ml}$ -82.0 $\mu\text{g/mg}$ for tartrazin. The concentration of all food dyes indicate a high value in all foodstuffs depending on the type or flavor of the products. These values correspond to the same nominal values as expressed by mg/100 ml when 5 g of sample and a final volume of 50 mL are considered. Data about the concentrations of dyes would be important instrument to help consumers in selecting more adequate products to avoid the intake of large amounts of additives, which can led to health problems, mainly in children. Quantification limits were far below the CODEX legislated values because in Iraq we don't have Standard Specification, (Table 1) satisfying this

legislation for the determination and control of these dyes in foodstuffs and soft drinks. some countries bounded using some of food dyes but some countries using these dyes till now because of their advantages such as high stability to light, oxygen and pH, color uniformity, low microbiological contamination, relatively lower production costs, etc [22]. This is important for specific groups of people, especially children, since hyper kinesis (a condition characterized by hyperactivity) has been shown to be related in several clinical studies [23] with the consumption of high levels of synthetic food colors. Finally the most consumer of these products are children they have more than one product in a day and this thing causes health problems for them

4. CONCLUSION

The concentration of five azo dyes were determined and detected in thirty five children food samples using HPLC technique, some of these samples indicates high concentration of Allura Red and Sunset yellow in soft drink, the range of their concentration far of the acceptable daily intake (ADI).

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