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Original Research Article

Determination of tartrazine in some food and medicine samples after solid phase extraction

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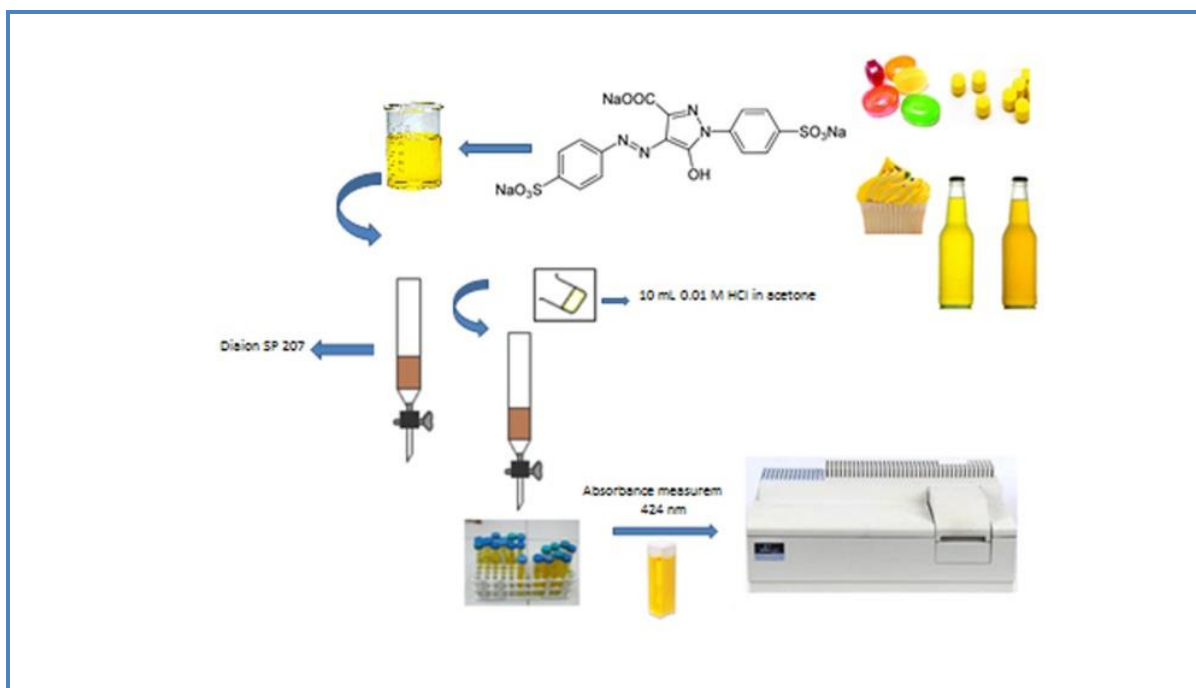
ABSTRACT

Food dyes are one of the most widely used additives in commercial food products such as beverages, candies, pharmaceuticals, and cosmetics. Despite the laws and regulations which aim to limit the synthetic colourant usage due to their pathogenic effects, they are widely used. Therefore, analyzing these dyes become very important for human health. Tartrazine is one of the commonly used synthetic food dyes and so the development of a new, simple, and accurate separation, preconcentration, and spectrophotometric determination of tartrazine (E 102) was the aim of this work. The method was based on solid-phase extraction by using Diaion SP-207 resin and determination with UV Spectrophotometer. Analytical parameters affecting the recoveries of tartrazine were investigated to find the optimal conditions. The retained tartrazine was removed from the column by 10 mL of 0.01 M HCl in acetone. The recovery values were found as >95%. By this new method, tartrazine contents of various food and medicine samples were determined successfully. The detection limit was found as 3.52 µg/L. Calibration standards used in the experiments were in the range of 10^{-7} - 1×10^{-5} mol L⁻¹ with a correlation coefficient of 0.999.

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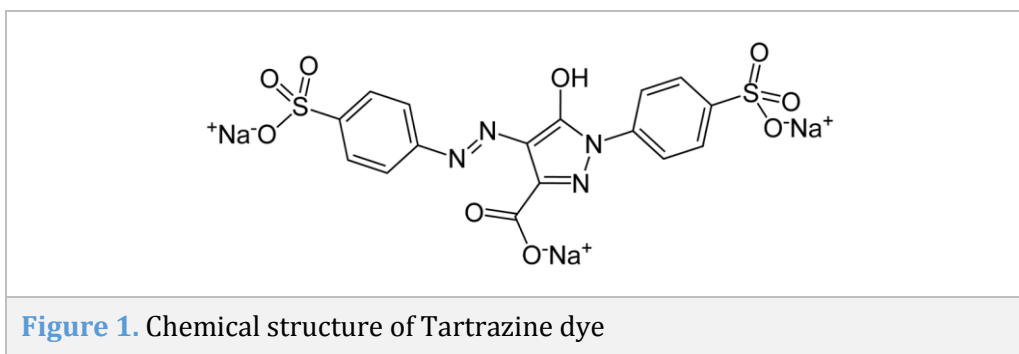
Graphical Abstract



Introduction

Food dyes either natural and synthetic are widely used in the food industry to enhance the aesthetic appeal of various food products. The colour is the most effective selection criterion for people to choose food. One of these dyes is

Tartrazine (Figure 1), a synthetic lemon yellow coloured, water-soluble mono azo pyrazolone dye, extensively used in the soft drinks, juices, candies, chewing gums cookies, ice creams and medical products. It is a dye derived from coal tar and also given the names as E102 or FD&C Yellow 5 or C.I. 19140 [1, 2].



Depending on how often the synthetic colourants are consumed they can cause health problems. To prevent such problems, there are laws and regulations to control the addition of synthetic colourants to foods. Therefore,

analyzing the synthetic colourants in food is very important, leading the researchers to focus on investigating the additives in food to assess their toxicity in the last years. The toxicological evidence for the synthetic colourants is

considerably greater compared to dyes produced naturally because of their chemical complexity [3-4].

More than 300 new works have been carried out on laboratory animals and clinical tests on human after the first safety regulation was regulated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1964. There are also limitations in Turkey to use tartrazine in food as an additive. According to Turkish Food Codex Food Additives Regulation (2013) tartrazine can be used at a maximum amount of 100 mg/L or 100 µg/kg in food, and it is mentioned there it can cause hyperactivity in children [5]. In many of the works done about tartrazine was incriminated in hypersensitivity reactions and it has been started questioning if it was safe or not [6]. The studies done in the last years revealed that, the tartrazine can cause some diseases such as allergies, asthma, and hyperactivity in childhood [7].

Synthetic dyes may also cause hazardous health effects when entered the food chains of humans and aquatic animals because of their toxicity as well as their byproducts. These synthetic dyes change the pH, colour and chemical oxygen demand of the water, preventing the growth of microbial organisms. Dyes may also change the photosynthesis process by blocking the entrance of sunlight. Thus, these dyes have to be removed from the wastewater before discharging them into aquatic systems [8]. Since tartrazine is a member of the azo class as a nitrous derivative, it is transformed into aromatic amine in the organism by reduction which causes sensitivity [9].

Several analytical methods have been used to determine the tartrazine such as HPLC [10-12], spectrophotometry [3, 13-16] and electrochemical methods [17-19]. Solid-phase extraction is a widely used method to separate and preconcentrate tartrazine before its

determination [20-23]. The presented study aims to develop a new separation-preconcentration technique for the spectrophotometric determination of tartrazine by using Diaion SP-207 as a solid-phase for the first time. Diaion SP-207 is a brominated styrene-divinylbenzene polymer with a hydrophobic nature, a mesh size of 20–60, a surface area of 650 m².g⁻¹, and a pore size of 105 Å [24].

Methods

Reagents and solutions

High purity reagents from Merck, Darmstadt were used in this work without further purification. Diaion SP-207 was purchased from Sigma, St. Louis, USA (Supelco no: 13623-U). Analytical grade tartrazine was obtained from E. Merck, Darmstadt. 100 µL⁻¹ tartrazine solution was prepared daily. All the solutions including diverse ions and dyes were prepared by using high purity compounds. Standard and model solutions were prepared daily by diluting the stock standards.

Instrument

A Perkin Elmer Lambda 25 UV-Visible spectrophotometer (USA) was used to measure the absorbance of tartrazine in the solutions at 424 nm. A Human Model RO 180 (Human Corp., Seoul, Korea) was used to water purification and production of water with a conductivity of 1 µS/cm. A Shimadzu Aux220 analytical balance (Shimadzu Corporation, Kyoto, Japan) was used for weighing.

Solid phase extraction procedure

Model solutions having a medium of 0.05 M HCl were prepared by adding 300 µg of tartrazine to test the method before using the method to analyze the contents of tartrazine in

real samples. A 15.0 cm long column made of glass having a diameter of 1.0 cm was prepared by filling with 500 mg of Diaion SP-207 resin was used in the experiments. At first, the column was conditioned by passing 3 mL of 0.05 M HCl before passing the model solution at a flow rate of 3 mL/min. After the model solution passed the column completely, 10 mL of 0.05 M HCl in acetone loaded to the column as the eluent solution and passed with a flow rate of 3.0 mL/min. Three replicate samples were prepared and used for every test working. 300 µg of tartrazine was added in a 50 mL beaker for preparing the model solutions and effects HCl concentration, eluent type, sample volume, flow rates of solutions and matrix effects were examined for the quantitative recoveries of tartrazine. The retained dye from the column was quantitatively recovered with 10 mL 0.01 M HCl in acetone. The absorbance of tartrazine in the solutions were measured by UV-Vis spectrophotometer at 424 nm.

Applications

Various candies, Turkish delight, drink powder and medicine samples sold in Nevşehir-Turkey were used to determine tartrazine. Before applying the preconcentration method,

drink powder, Turkish delight, and candy samples were tared, and distilled water was used to dissolve them. Centrifugation process was applied to remove the dissolved residuals if needed. 1 g of each medicine sample was prepared for analysis by dissolving in water and diluting to 50 mL and filtering with cellulose nitrate membrane filter (Osmonics, Westborough, MA). Then, the proposed preconcentration method was applied to 5 mL of each prepared samples.

Results and Discussions

The parameters expected to influence the recoveries of tartrazine such as the concentration of HCl, eluent type and volume, sample volume, flow rates of sample and eluent solutions and matrix effects were optimized.

Influences of HCl concentration

The model solutions were prepared at the HCl concentrations in the range of 0.01-0.5 M to see how the HCl concentration affects the recovery of tartrazine. According to the results presented in Figure 2, the HCl concentration was selected as 0.05 M for further experiments since the highest preconcentration factor was gained by using it.

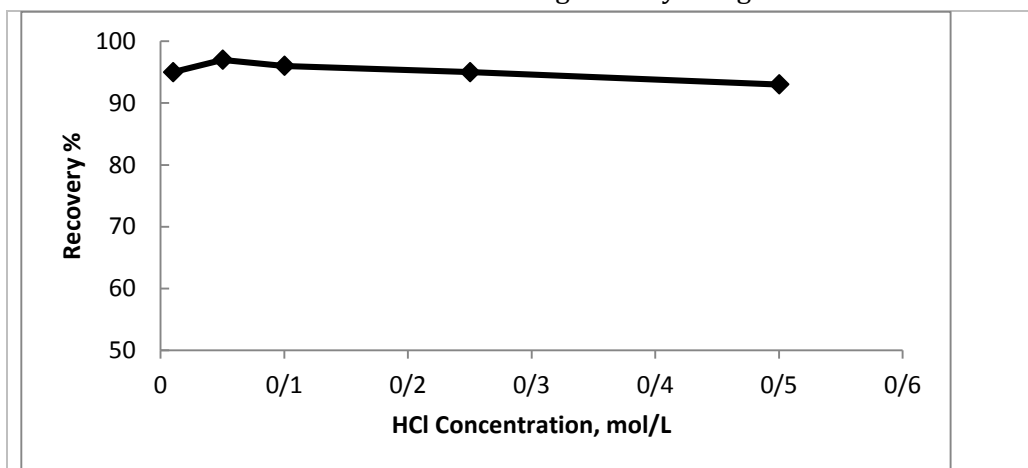


Figure 2. Effect of HCl concentration on the recovery of Tartrazine (N= 3)

Eluent type and volume

Desorption of the analyte is also as important as the adsorption of the analyte in preconcentration studies to get high preconcentration factors. Therefore, to find the

optimum eluent solution various solutions were tried to desorb the retained tartrazine from the column packed with Diaion SP-207 resin. The results are given in Table 1. 10 mL of 0.01 M HCl in acetone was used as the eluent for further studies.

Table 1. Effect of eluent type and volume on the recovery of Tartrazine (N=3)

Eluent type	Eluent Volume, (mL)	Recovery %
Acetone	5	80±3
Acetone	10	93±2
Ethanol	10	53±2
DMFA	5	94±2
DMFA	10	109±3
DMS	10	83±3
HCl in Acetone	5	98±1
HCl in Acetone	10	100±1
HCl in Ethanol	5	72±3
HCl in Ethanol	10	96±2

Effect of flow rate

Controlling the flow rates of the sample and eluent solutions while passing through the column is another effective parameter in preconcentration studies. In the column experiments solutions passes from the column by controlling its stopcock to ensure the desired

flow rates. In this study, sample solution and eluent flow rates were examined in the range of 1.0-6.0 mL/min to identify the optimum flow rates. The results are shown in Figure 3 and Figure 4 for sample and eluent solutions individually, and the quantitative recoveries were obtained in 1.0-5.0 mL/min flow rate range.

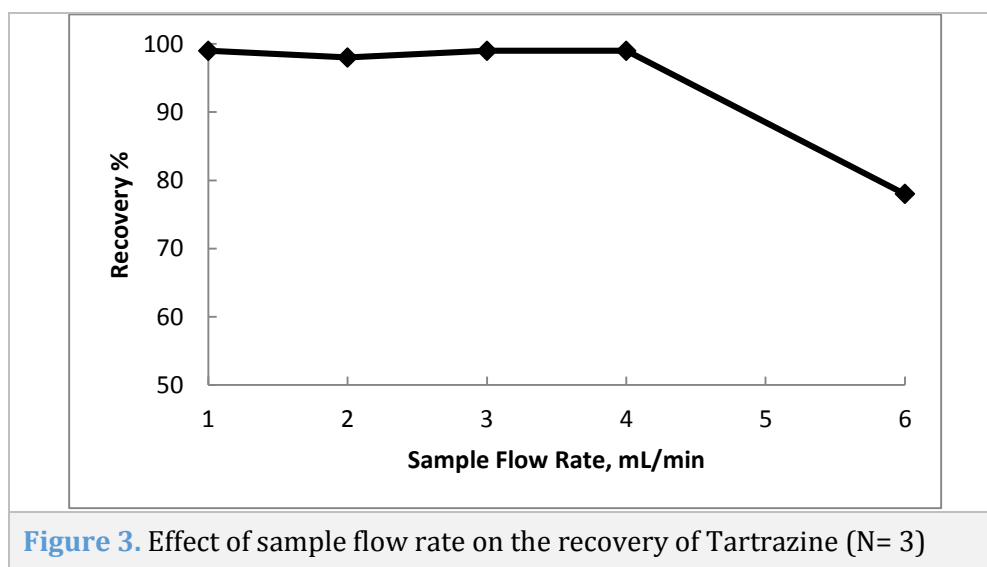
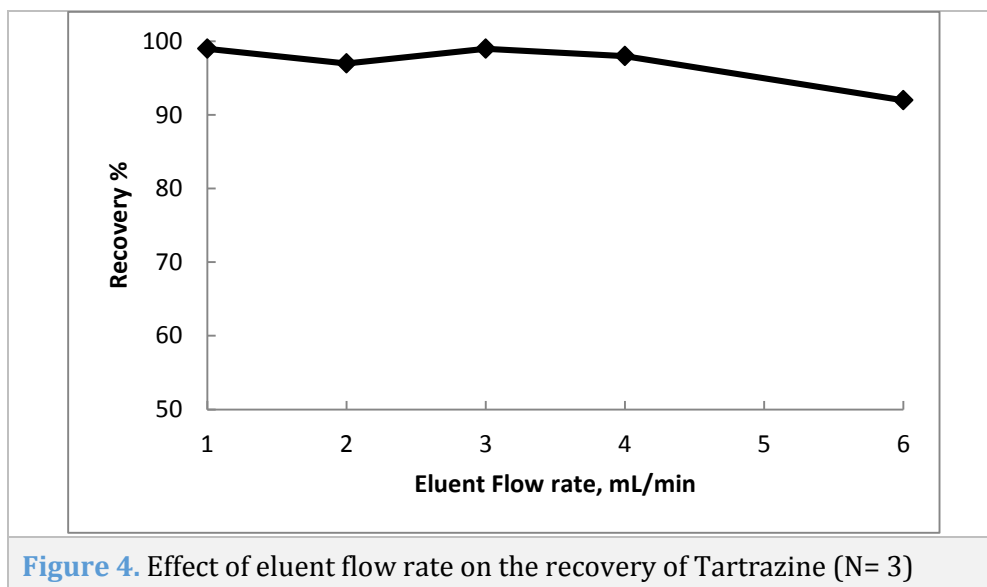


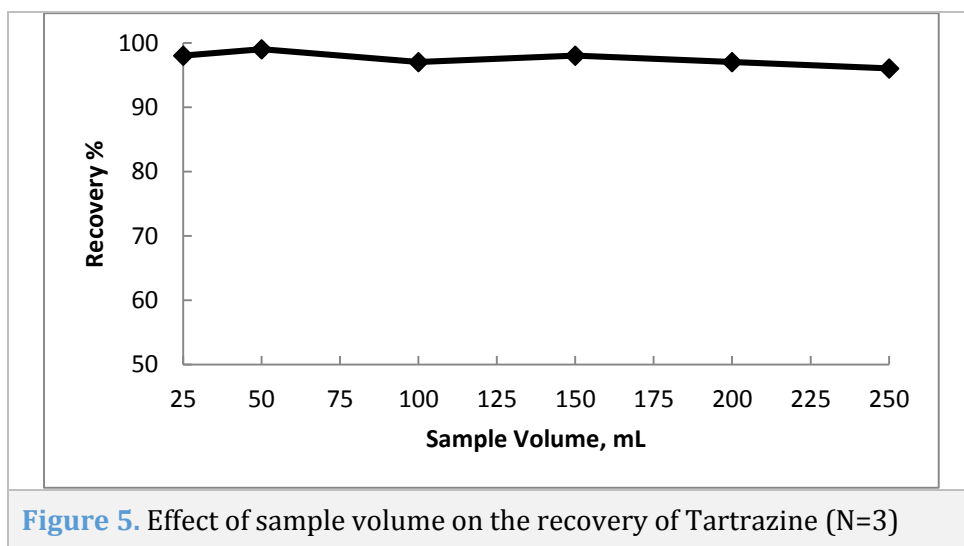
Figure 3. Effect of sample flow rate on the recovery of Tartrazine (N= 3)



Effects of volume

Model solutions containing 300 μg of tartrazine were prepared in volumes of 25–250

mL to investigate the effect of volume. As seen in [Figure 5](#), the tartrazine was quantitatively recovered in all sample volumes that were studied.



Matrix effect

The accurate and precise analysis of the analytes within their coexisting matrices is very necessary for the preconcentration studies. Thus, to see the effects of some ions and some other food dyes on the recoveries of tartrazine the presented method was applied to the model

solutions containing the variety of matrix ions and dyes. The recovery results found for the examined ions and the dyes are listed separately in [Table 2](#) and [Table 3](#). According to the results presented in [Table 2](#) and [3](#), the method can be applied to the analysis of tartrazine without any interference of the studied ions and dyes.

Table 2. Matrix effect on the recovery of Tartrazine (N=3)

Ion	Concentration (mg/L)	Added As	Recovery %
Na ⁺	1000	NaCl	103±2
Mg ²⁺	500	Mg(NO ₃) ₂	97±1
K ⁺	1000	KCl	101±2
Ca ²⁺	500	CaCl ₂	97±2
NO ₃ ⁻	100	NaNO ₃	100±1
SO ₄ ²⁻	1000	Na ₂ SO ₄	98±2
CO ₃ ²⁻	100	Na ₂ CO ₃	101±1

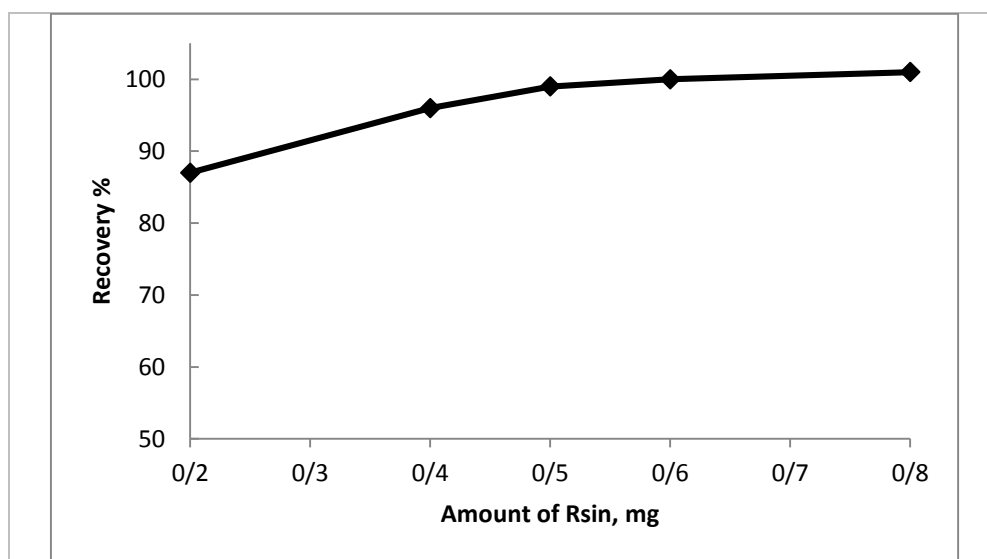
Table 3. Effects of some other food dyes on the recovery of Tartrazine (N=3)

Added Dyes	Recovery %
Allura Red	97±2
Carmin	97±1
Brilliant Blue	99±2
Carmoisine	102±3
Lime Green	100±1

Effects of amount of Diaion SP-207

Diaion SP-207 resin amount was tested in the range of 0.2-0.8 g to see the effect of the solid

phase amount on the recovery of tartrazine. [Figure 6](#) reveals that, the tartrazine was recovered quantitatively when 0.4-0.8 g Diaion SP-207 resin was used to fill the column.

**Figure 6.** Effect of resin amount on the recovery of Tartrazine (N=3)

Analytical performance

The accuracy of the proposed method was evaluated by adding different amounts of

tartrazine in a medicine sample purchased from Nevsehir-Turkey in the range of 0-40 µg, then applying the presented method given in the experimental part. The added and measured

tartrazine amounts found to be compatible with each other (Table 4). Therefore, the presented method can be successfully applied for determining the tartrazine in real samples. The detection limits were calculated by applying the

method on 15 blank samples and found as 3.52 µg/L. It was the concentration obtained from three times the standard deviation of these blank solutions.

Table 4. Tests of addition/recovery for the application of the method on a medicine sample containing Tartrazine (N=3)

Analyte	Added (µg)	Found (µg)	Recovery %
Tartrazine	0	11.7±1.4	-
	10	21.9±1.3	98±0
	20	33.0±0.9	97±1
	40	52.9±3.4	95±3

Real sample analysis

After obtaining the optimal experimental conditions for the proposed preconcentration/separation method, various samples such as Turkish delight, medicine and

drink powders were gathered from markets in Nevşehir (Turkey) to apply the method for the determination of their tartrazine contents. The results of the analysed samples by the presented method are presented in Table 5.

Table 5. Analysis of Tartrazine containing samples (N=3)

Tartrazine Containing Samples	Concentration (µg/g)
Cake Decoration Candy (A)	150.5±14.7
Yellow Colored Turkish Delight (B)	36.1±2.3
Green Colored Turkish Delight (C)	241.8±13.3
Banana Aromated Pudding Powder (D)	35.3±5.2
Powder Drink Peach (E)	1349.6±157.3
Drink Powder Lemon (G)	385.6±11.0
Medicine (H)	5433.1±479.4
Medicine (K)	499.7±79.8
Medicine (L)	869.9±7.0

Conclusion

This work reports a new, easy and affordable method for determining the tartrazine in food and medicine samples accurately and precisely after preconcentration and separation. The presented method is not affected by the matrix components present in the samples. The findings of the method revealed that, it can be effectively applied to the analysis of tartrazine in samples such as drink powder, Turkish delight and medicine. When the results of the analyzed samples are compared with the limits given in Turkish Food Codex Food Additives

Regulation, tartrazine found in the medicine samples are 5-50 times the maximum value, which is 100 mg/kg. Only two Turkish delight samples are less than the limit value while the powder drink samples and pudding powder exceed the limit. In the Regulation 02008R1333-EN-20.03.2017-032.001, the permitted limit for tartrazine is also 100 mg/kg or 100 mg/L in bitter soda and some food samples, and it is required to be stated in the label of the product that tartrazine may have harmful effects on the activity and attention in children. So, consumers of such products

containing tartrazine should be careful especially if the subject is children.

Acknowledgement

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Conflict of Interest

Authors declare that they have no conflict of interest.

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