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Identification of Synthetic Food Dyes in Various Candies

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Abstract: The aim of this research is to analyze various synthetic dyes in different candies which are a very big jeopardy for human health. The research was made on different colored candies of different companies. The synthetic dyes were analyzed by TLC technique. The dye that is consumed the most is apple green 28.5%. 14.2% of the samples were found to have no synthetic dye. Whereas 85.7% of the samples contains different synthetic food dyes. People should realize the harmful effects of these dyes and evaluation of these dyes should be done.

Keywords: Synthetic Food Dyes, Candies, TLC, Harmful Effects.
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Introduction

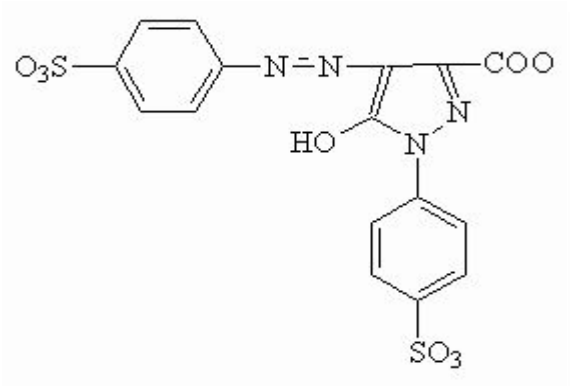
Food dye can be any chemical substance or pigment that will impart color to our food. The main purpose to add a food dye is to give good appearance to food stuffs[1]. It can be classified in two major groups, i.e. natural dyes and synthetic dyes. In the early times colors were used to dye fabrics which were extracted from minerals, plants and animals, these dyes are categorized as natural dyes. After the production of these dyes a new class of dyes were produced which were cheaper, easy to apply and gives color more quickly than the natural dyes these are named as synthetic dyes.

First synthetic dye, mauve[2], was discovered by Sir William Henry Perkins in 1856. As the time passed more synthetic dyes were developed, the use of these dyes in food makes them attractive, avoid color loss due to environmental elements, and also add colors to the colorless food including these advantages there are more benefits of using these dyes as they are cheap, highly stable to heat, light and oxygen, gives uniform color and show low microbial contamination[3].

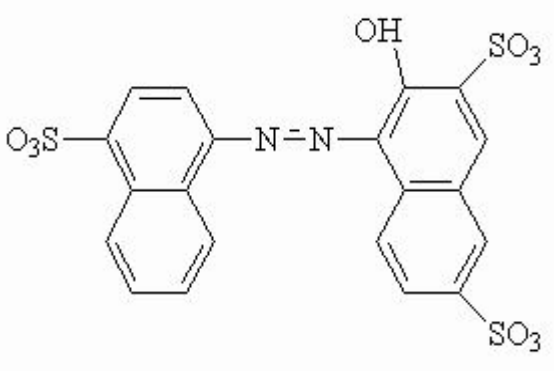
These dyes have great ability to bind chemically to proteins in wool, silk, fiber and cells. Different nature of dyes differs in their ability to bind with different materials. Natural colors are healthier than synthetic colors due to the fact that natural colors are harmless to human health and they must be used in food stuffs rather than synthetic dyes but instead of these advantages there are also some disadvantages of natural dyes which show that they are expensive, decompose easily, have shorter shelf life but the reason behind importance of using natural dyes is that synthetic colors cause carcinogenicity, hyperactivity in children, weakening of immune response, some dyes are reported to induce tumors in mice and also in some cases vitamins deficiency, which are not reported after using natural dyes[4]. After extensive work on these dyes, they were classified into five groups which include azo, triarylmethane, quinolone, xanthene, and indigoid compounds on the basis of their structure[5].

For the welfare of human health there must be some distinction between the dyes which should not be used in food and are carcinogenic following this, some dyes are banned which are harmful when consumed they include Amaranth, E123, FD&C Red No. 4, FD&C Red No. 32 was used to color Florida oranges, FD&C Orange Number 1,FD&C Yellow No. 1, 2, 3, and 4, FD&C Violet No. 1. There are also some food colors that are approved and can be used for human consumption Quinoline Yellow, E122: Carmoisine, E124: Ponceau 4R,

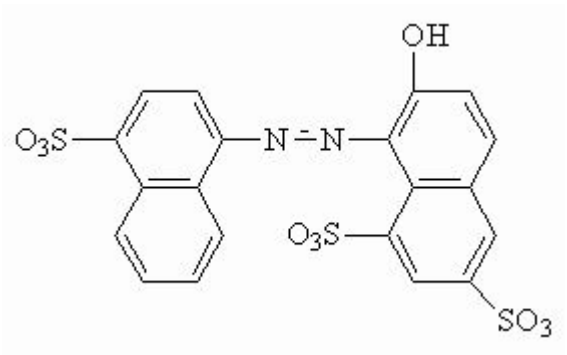
E131: Patent Blue V, E142: Green S[6]. The technique which we used for the determination of synthetic dyes is thin layer chromatography. This best chromatographic technique which can be used for qualitative as well as quantitative determination of synthetic food dyes because of it is simple, economic, quick and precise method as well as satisfactory results can be obtained [7].



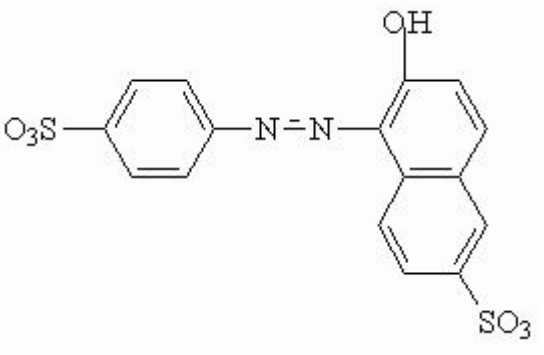
E 102 Tartrazine



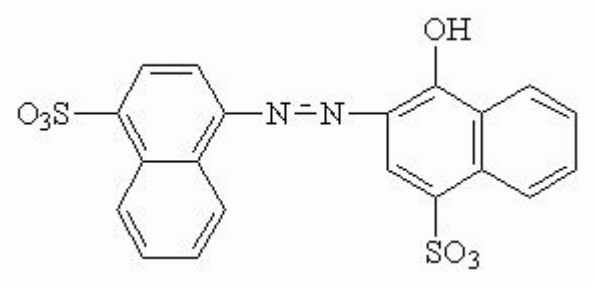
E 123 Amaranth



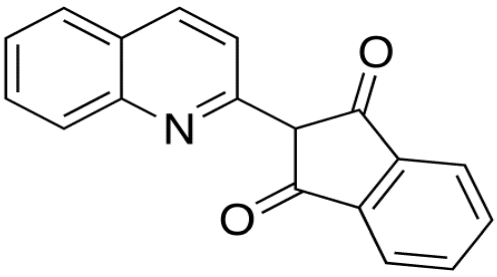
E 124 Ponceau 4R



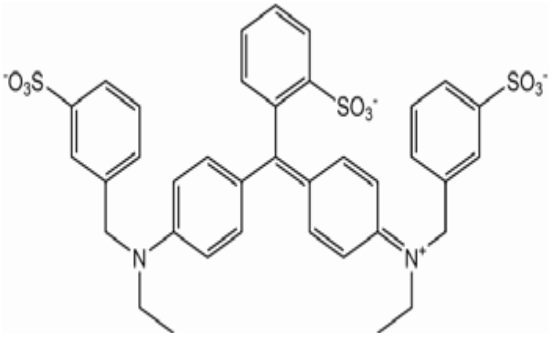
E 110 Sunset Yellow

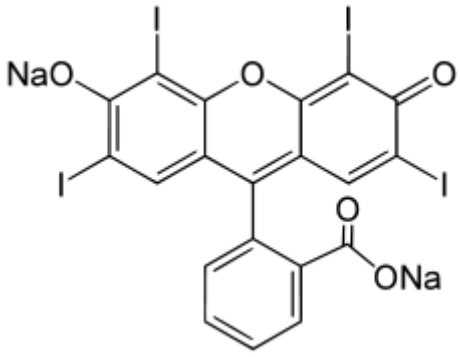


E 122 Azorubine E 104

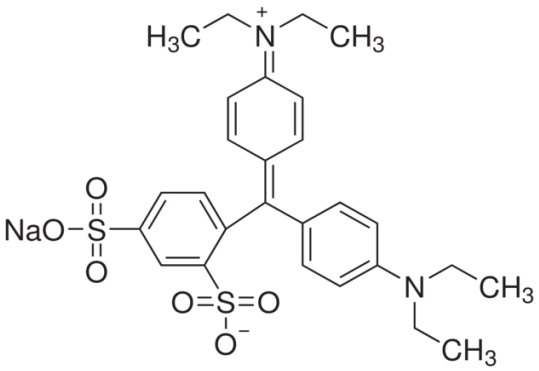


Quinoline Yellow





E 133 Brilliant Blue **E 127 Erythrosine**



E131 Patent Blue

Figure 1. Structures of various synthetic food dyes [8]

MATERIALS AND METHODS

Reagents

Synthetic food dye solutions which serve as standard solutions. 5% glacial acetic acid, 1M ammonia, 2-propanol and ammonia solution were used.

Standards used

1. Tartrazine
2. Raspberry red
3. Lime juice
4. Erythrosine
5. Ponceau
6. Red
7. Sunset yellow

Preparation of mobile phase

Ammonia (20mL) and 2-Propanol (80mL) are mixed together.

Thin Layer Chromatography

Preparation of samples is a vital phase of analysis that has a large impact on the interpretation of results. 15 samples of different candies were analyzed. Each candy sample weighing at least 36 gram was taken randomly and grinded in pestle and mortar. Grinded candies were dissolved in 50mL of 5% glacial acetic acid in 250mL conical flask. Shake the solution vigorously or put the solution on shaker for 10-15 minutes. For TLC analysis the solution must be purified from all the impurities such as fats, sugar or other particles that may cause hindrance tothese synthetic dyes analysis. Filter the solution by Whatmann filter paper. Repeat the same process for the all other samples which are left. 50mL of the solution was then treated under the process recommended[9].

Purification and separation of food dye

Place 50mL of sample solution with 15-20 wool yarns in a 250mL glass beaker on hot plate for almost 1 hour. Under acidic conditions, synthetic food dyes were fully adsorbed by wool yarns leaving the solution almost colorless. The wool yarns are than comprehensively washed with cold water to remove other food elements leaving only the synthetic dye on the wool yarns. After the accomplishment of this step, next step is to add 30mL of 1M ammonia solution to the wool yarns and placed on hot plate again for 30-60 minutes. Under basic conditions, synthetic food dyes were separated. The wool yarns that adsorbed synthetic food dyes were now colorless and were discarded. The solutions were evaporated until the point where only synthetic food dyes were left probably along withsome food particles that can be removed by again adding 50mL water, 1mL acid and wool yarns.

Staining of TLC plates

Activated silica gel serves as stationary phase whereas the mobile phase was ammonia and 2-propanol (80:20). Silica gel was activated by placing the TLC plate in incubator at 90-100°C for 8-10 min. First of all the plate was sorted horizontally with the help of pencil from one direction and approximately 3cm apart from each other. Each point is labelled above it with the names they were assigned. The next step will be staining. A very small amount of the separated synthetic food dyes was taken in a capillary tube and the plate was stained with it. The stain of each color should not be more than 2-3mm in diameter. Staining of a single dye can be repeated 2-3 times if dye is low in density in the solution. Standards were also stained on the plate along with the synthetic food dyes for their comparison and identification. Let the stains dry at room temperature for 3-5 minutes.

Preparation of TLC tank

First of all TLC tank is washed prudently and dried. 20mL of 2-propanol and 80mL of ammonia are poured in TLC tank and stirred. The TLC plates were placed in the tank until the solvent travels the maximum distance. Cover the TLC tank.

Figure 2. Various steps for identification of synthetic dyes in candies

Sample Labels	Retention factor = Rf		
	Distance travelled by solute = Ds (cm)	**Distance travelled by solvent front = Df (cm)**	**Rf = Ds/Df**
A	5.2	8.2	0.73
B	5.2	8.2	0.63
C	5.8	6.9	0.84
D	6	8.4	0.71
E	5.3	8.4	0.63
F	No synthetic dye was found		
G	No synthetic dye was found		
H	5.1	8.4	0.60
I	5.8	8.4	0.61
J	5.8	6.9	0.84
K	5.3	6.9	0.768
L	5.3	6.9	0.768
M	5.9	6.9	0.855
N	6.1	6.9	0.88

Table 1. Rf values of different food dyes extracted from candies

RESULTS AND DISCUSSION

Rf value is referred to as the distance travelled by the sample synthetic food dye in relation to the distance travelled by the standard dye. The distance travelled by the synthetic food dye can be swayed by different environmental conditions such as temperature, atmospheric pressure, air flow and freshness of eluent[10]. At the end, the type and name of the sample dyes were identified with respect to the distance travelled by them. In table 1 Rf values of different dyes in candies is calculated. In most of the candies apple green dye was found. There were two candies named as Hajmola and Butter Scotch in which no

synthetic dye was found. Butter scotch was caramel colored candy at first, but its color was may be due to its sugary content, whereas sample H contains a dye which was a mixture of three different synthetic dyes.

Table 2. Comparison of the extracted yes with different standards

Standards	**Samples**														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Panceau		✓													
Raspberry red									✓						
Red No. 4															
Tartrazine															
Brown								✓							
Apple green										✓	✓	✓			
Focol apple green										✓	✓		✓		
Sunset yellow	✓			✓											
Amaranth															
Lime juice			✓											✓	
Erythrosine															
Colorless						✓	✓								

From the above results, we can infer that 14.2% candies contain no synthetic food dye. The color was may be due to its sugar contents or any fabric dye or inadequate dye was used. The other candy F, contains no synthetic dye and it was not found harmful for human consumption. In one candy labelled as E, a mixture of three synthetic dyes was used and this candy shouldn't be marketed. It may lead to certain health risks. The synthetic dyes may react with each other causing certain health hazards.

Table 3. Comparison of the dyes identified and the name mentioned on their wrapper

Sample Name	Dye of the candy mentioned on their wrappers	Dyes identified by TLC
A	Not mentioned	Sunset yellow
B	Not mentioned	Panaceau
C		Lime juice
D	Not mentioned	Sunset yellow
E	Not mentioned	Mixture of colors
F	Not mentioned	No synthetic dye detected
G	Tartrazine, Brilliant Blue FCF and Brown	No synthetic dye detected
H		Brown
I	Azorubine	Raspberry red
J	Tartrazine and Brilliant Blue FCF	Apple green
K	Green	Apple green
L	Dark Green	Apple green
M	Apple Green	Apple green
N	Not mentioned	Lime juice

Most of the samples contain Apple green synthetic dye. So we may surmise from these results that apple green might be most commonly used dye. FDA is anxious for human health related to food safety and has issued various testing procedures for this purpose. In 2008 the Center for Science in the Public Interest (CSPI) in Washington, DC, petitioned the Food and Drug Administration (FDA) to ban artificial food dyes because of their connection to behavioral problems in children.

Neurotoxics effects are increasing day by day and hence it is a point to ponder[11]. Pharmacokinetics data can be very helpful in food safety, food colorsand toxic effects in animals to humans and it also provides vision of the mechanism of toxicity[12]. Quinoline blue and brilliant black cause genotoxicity in human lymphocytes[13]. Amaranth, water-soluble red color, is confirmed to cause carcinogenicity in mice[14]. Evaluation of the development of toxicity due to consumption of annatto in the rat showed that it has no adverse effect. Tartrazine which is a commonly used dye is now known to cause hyperactivity, urticaria, skin rashes and intolerance to aspirin[15].Tartrazine is also known to effect behavioral development, exploratory behavior, maze learning, reproductive toxicity, DNA damage in gastrointestinal cells, increased number of eosinophils and lymphocytes in mice[16-18]. Canthaxanthin, is a keto-carotenoid orange-red pigment widely distributed in nature, when deposited in retina, cause retinopathy[19].

CONCLUSION

From the above discussion we can comprehend the following conclusion that synthetic dyes are harmful for human consumption and there must be a proper check on the determination and appraisal on the intake of synthetic food dyes. However, natural dyes can also have toxic effects on humans but synthetic dyes are more toxic. Children are hypothetically more vulnerable to be effected by these synthetic dyes as these dyes are most commonly ingested by them in candies, pastries, soups, jellies, etc. There must be an organization for regular assessment of these dyes in Pakistan. If the synthetic dyes are replaced by non-emulsion based water soluble food dyes or second generation natural food dyes namely cap color the toxic effect of these dyes would be lessened. TLC is a best solution for qualitative analysis of food dyes as it gives reasonable results in a very short time span and requires less complex apparatus and methodology as compared to other chromatographic techniques.

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