CHAPTER 1

INTRODUCTION

A hard candy or boiled sweet is a sugar candy prepared from one or more sugar based syrups that is boiled to temperature of 160◦C to make candy. Among the many hard candy varieties are the stick candy such as the candy cane, lollypops, aniseed twists. Sugar candies include hard candies, soft candies, caramels, marshmallows, taffy, and other candies whose principle ingredient is sugar. Commercially, sugar candies are often divided into groups according to the amount of sugar they contain and their chemical structure. Physically, candy is characterised by the use of a significant amount of sugar or sugar substitutes. Unlike a cake or loaf of bread that would be shared among many people, candies are usually made in smaller pieces.

However, the definition of candy also depends upon how people treat the food. Unlike sweet pastries served for a dessert course at the end of meals. Each culture has its own ideas of what constitutes candy rather than dessert. The same food may be a candy in one culture and a dessert in another. (13)

Food colour or colour additives is any dye, pigment or substance that impacts colour when it is added to food or drink. They come in many forms consisting of liquids, powders, gels and pastes. Food colouring is used both in commercial food production and in domestic cooking. Food colorants are also used in variety of non food applications including cosmetics, pharmaceutical, home craft projects, and medical devices.

Food colouring is a tactic the food industry has utilised for decades. The dangers of food colour are often an issue in food safety, with many claiming them to be toxic and factor to the rise of ADHD (Attention Deficit Hyperactivity Disorder) in recent years. Curiously enough, many natural colours previously used to colour food contained toxins such as mercury and at the turn of the 20th century, companies began to create synthetic solutions to replace harmful natural dyes.

Some most frequently used food colours:-

Blue: brilliant blue, indigo carmine

Red: allura red, erythrosine

Yellow: tartrazine, sunset yellow

Green: fast green.[12]

Colour adulteration is the most frequent form of adulteration. Use of any food colour prohibited under the prevention of food adulteration act in or upon and food or beverage. Use of marketed colours not stamped with the ISI mark of quality. Colours are not foods and do not add to the nutritive value of food. Colours severs to mask defects I food making inferior foods look superior. Colouring are high risk for children and the foetus in a pregnant mother. Colourings may react it the food and of change to poisons in the body, causing mutation cancer or other toxic effects. [1]

The amount of food colour like sunset yellow, brilliant blue and tartrizine have been tested and estimated from the sample like Poppins a brand of Parle. The food colours like sunset yellow, brilliant blue and tartrazine if it is used more than the limited amount for a long time it may cause chromosomal damage, tumours, allergies etc. All these colours are made of elements like carbon, hydrogen, nitrogen, sodium and oxygen.

People associate certain colours with certain flavours and the colour in the from candy to wine. Sometimes the aim is to simulate a colour that is perceived by the consumer as natural such as adding red colouring to glace cherries (which would otherwise be begei), but sometimes it for effect, like the green ketchup that Heinz launched in 1999. Colour additives are used in foods for many reasons including.

Colour additives are used in foods for many reasons includes to make food more attractive, appealing, appetizing and informative. Offset colour loss due to exposure to light, air , temperature extremes, moisture and storage conditions. Correct natural variations in colour. Enhance colours that occurs naturally. Provide colour to colourless and fun foods. Allow consumers to identify products on sight, like candy flavours or medicine dosages.

Widespread public belief that artificial food colouring causes ADHD- like hyperactivity in children originated from Benjamin Feingold, a pediatric allergist from California, who proposed in 1973 that salicylates, artificial colours and artificial flavours cause hyperactivity In children. However there is no evidence to support broad claims that food colouring causes food intolerance and ADHD- like behaviour I children.

Brilliant Blue FCF (Blue 1) is a synthetic organic compound used primarily as a blue colourant for processed foods, medications, dietary supplements, and cosmetics. It is classified as a triarylmethyl dye and is known under various names, such as FD&C Blue No. 1 or Acid Blue 9. It is denoted by E number **E133** and has a colour index of 42090. It has the appearance of a blue powder and is soluble in water and glycerol, with a maximum absorption at about 628 nanometers. It is one of the oldest FDA-approved colour additives and is generally considered nontoxic and safe.

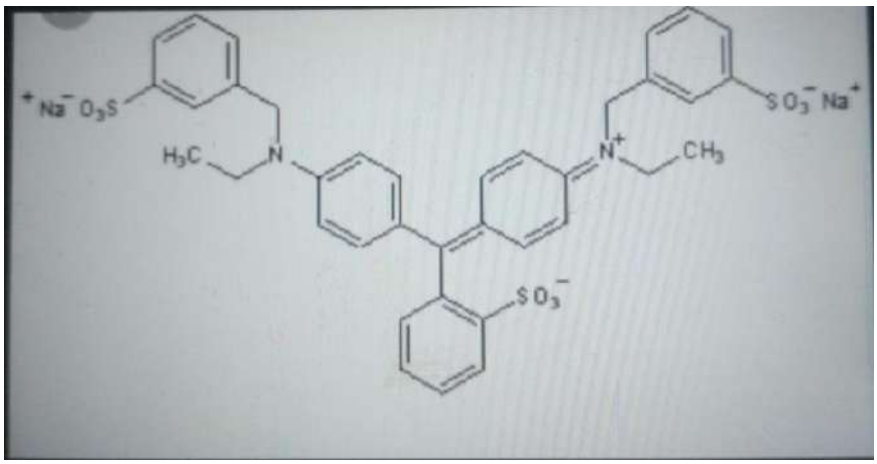


Fig. 1. Chemical structure of brilliant blue [15]

The dye is poorly absorbed from the gastrointestinal tract and 95% of the ingested dye can be found in the faces. When applied to the tongue or shaved skin, Brilliant Blue FCF can be absorbed directly into the blood stream. Due to its nontoxic properties, Brilliant Blue FCF has been used as a biological stain. When dissolved in an acidic medium, this dye has been used to stain cell walls, bacteria, and fungal cells. The dye does not inhibit the growth of any of these species sunset Yellow FCF (also known as Orange Yellow S, or C.I. 15985) is petroleum -derived orange azo dye with a pH dependent maximum absorption at about 480 nm at pH 1

and 443 nm at pH 13 with a shoulder at 500 nm. When added to foods sold in the United States it is known as FD and C Yellow 6; when sold in Europe, it is denoted by E number E103.

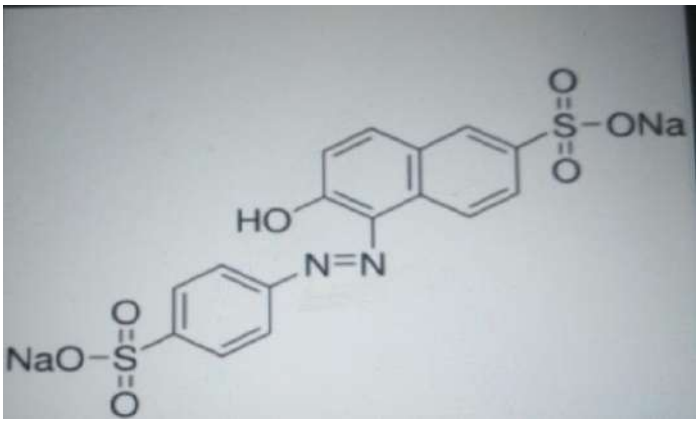


Fig. 2 Chemical structure of sunset yellow.[16]

The acceptable daily intake (ADI) is 0–4 mg/kg under both EU and WHO/FAO guidelines. Sunset Yellow FCF has no carcinogenicity, genotoxicity, or developmental toxicity in the amounts at which it is used.

It has been claimed since the late 1970s under the advocacy of Benjamin feingold that Sunset Yellow FCC causes food intolerance and ADHD -like behavior in children but there is no scientific evidence to support these broad claims. It is possible that certain food coloring may act as a trigger in those who are genetically predisposed, but the evidence is weak.

Tartrazine is a synthetic lemon yellow azo dye primarily used as a food colouring. It is also known as E number E102,C.I. 19140, FD AND C Yellow 5, Acid Yellow 23, Food Yellow 4, and trisodium 1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-5-pyrazolone-3-carboxylate).

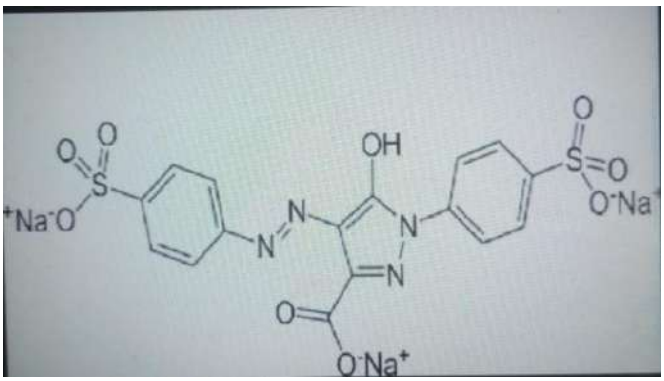


Fig. 3. Chemical structure of tartrazine.[17]

Tartrazine is a commonly used colour all over the world, mainly for yellow, and can also be used with Brilliant blue FCF (FD&C Blue 1, E133) or Green S (E142) to produce various green shades.

Many foods contain tartrazine in varying proportions, depending on the manufacturer or person preparing the food. When in food, tartrazine is typically labelled as "colour", "tartrazine", or "E102", depending on the jurisdiction, and the applicable labelling laws .Products containing tartrazine commonly include processed commercial foods that have an artificial yellow or green colour, or that consumers expect to be brown or creamy looking. It has been frequently used in the bright yellow colouring of imitation lemon filling in baked goods. The following is a list of foods that may contain tartrazine.[12]

CHAPTER 2

LITERATURE REVIEW

A Hussain, W sawaya, A Al Omair, S. Al Zenkin, H. Al Amiri , N Ahmed and M. Al Sinan (2005) estimated of dietary exposure of children to artificial food colours in Kuwait .The determination of colour additives in 344 foods items consumed was performed using high-performance liquid chromatography with diode array detector. A comparison with the Food and Agriculture Organization and World Health Organization acceptable daily intakes (ADIs) was undertaken to evaluate the potential risk associated with the consumption of artificial colour additives by children in Kuwait. The results indicated that out of nine permitted colours, four exceeded their ADIs by factors of 2–8: tartrazine, sunset yellow, carmoisine and allura red. Further, follow-up studies to provide insight into potential adverse health effects associated with the high intakes of these artificial colour additives on the test population are warranted.

Meenakshi Tripathi, Subash K. Khanna, Mukul Das (2005) made a surveilliance on use of synthetic colours in eatables (lucknow) vis a vis prevention of food Adulteration Act of India. Of the total 1199 analyzed samples, 69% coloured eatables revealed the presence of permitted colours while 31% samples contained non-permitted colours. samples of crushed ice which are preferentially consumed by children population, the presence of Sunset Yellow FCF and Tartrazine was found to exceed the permissible limit by 8 and 20 times while in rural areas Sunset Yellow FCF, Tartrazine and Carmoisine exceeded the permissible limit by 23, 16 and 15 times, respectively. Non-permitted colours such as Rhodamine B, Metanil Yellow, Orange II, Malachite Green, Auramine, Quinoline Yellow, Amaranth and Sudan dyes were identified in various foodstuffs.

Frank E Lancaster and James F Lawrence(2009) investigated presence of glucose and sucrose by thermal decomposition of the food colours amaranth, sunset yellow FCF tartrazine Mixtures of food colour, sucrose and glucose were heated at several

temperatures under simulated candy making conditions. Amaranth (128 μg/g), sunset yellow FCF (113 μg/g) and tartrazine (116 μg/g) were found to decompose by up to 25%, 10% and 22% respectively over a temperature range of 120–162°C. The decomposition increased with increasing temperature. The products found as a result of amaranth decomposition corresponded to naphthionic acid and amino-R-salt. No increase in intermediates was observed in the candies as a result of the decomposition of sunset yellow FCF and tartrazine during the cooking process.

Laura M. konig and britta renner (2007) explored meal colour variety and its consumption of food. In order to explore a colourful equals healthy association, the present study examined 486 real-life meal choices recorded by 108 participants. Participants recorded their lunch meals via mobile visual food recording, indicated the perceived meal colour variety, and added a short meal description using smartphone based ecological momentary assessment. Hence, eating colourfully seems to be a promising avenue for promoting a more intuitive but also healthy food choice strategy in consumers.

M. Cecilia F.Toledo, Monica S. Guerchon and Sidnei Ragazzi(2009) in Brazil determined potential weekily intake of artificial food colours by 3- 14-year- old children. Coloured food consumption data were obtained through dietary recall interviews and collection of the packages and/or labels of the coloured foods consumed during a two-week period. The results showed that all artificial colours used in the composition of 83 commercial food products, including jellies, juices, soft drinks, syrups and 57 different candies, were permitted for use in food in Brazil the year the survey was conducted (1986), in amounts below those prescribed by law. Comparison of the estimated potential intakes with the toxicologically Acceptable Daily Intake (ADI) showed that consumption of Amaranth, Sunset Yellow, Indigotine and Tartrazine by all children in the study represented approximately 24%, 3%, 0.05% and 0.4%, of the actual ADI values, respectively.

Charles Spence(2015) researched on the physiological impact of food colour. Colour is the single most important product-intrinsic sensory cue when it comes to setting people's expectations regarding the likely taste and flavour of food and drink. To date, a large body of laboratory research has demonstrated that changing

the hue or intensity/saturation of the colour of food and beverage items can exert a sometimes dramatic impact on the expectations, and hence on the subsequent experiences, of consumers By gaining a better understanding of the sensory and hedonic expectations elicited by food colour in different groups of individuals, researchers are coming to understand more about why it is that what we see modulates the multisensory perception of flavour, as well as our appetitive and avoidance-related food behaviours.

Wajih Sawaya, Adnan Hussain, Fawsiya Al-Avadhi, Naal Al-Hamad, Basma Dashti, Jameela Al-Saqger(2007) determined the consumption pattern of artificially coloured foods among children in Kuwait. Of 450 coloured foods available in the market, 344 that were commonly consumed by children were purchased from different co-operative societies and supermarkets distributed in Kuwait and were grouped into nine categories, namely: biscuits, cake, candy, chips, chocolate, drinks and juices, chewing gum, jelly, and lollypops. These were then analysed for their contents of artificial colour additives using a high-pressure liquid chromatography with diode array detector. The similarity in the high daily intake of drinks among children in Kuwait and other countries indicates a need to improve the diets of Kuwaiti children.

Bakthi Petigara harp, Enio Miranda-Bermudez, Carolina I.Baron, and Garald I. Richard (2012) in US determined the quantitatively tested whether the permited amount ofcolour additives in food products. The method involves extracting the colour additives from a product and isolating them from non-coloured components with a C_{18} Sep-Pak cartridge. The colour additives are then separated and identified by liquid chromatography (LC) with photodiode array detection, using an Xterra RP18 column and gradient elution with aqueous ammonium acetate and methanol. Limits of detection (LODs) ranged from 0.02 to 1.49 mg/l. This qualititative LC method supplements the visible spectrophotometric and thin-layer chromatography methods currently used by the USFDA's district laboratories and is less time-consuming and requires less solvent compared to the other methods. The extraction step in the new LC method is a simple and an efficient process that can be used for most food types.

Alison Downham and Paul Collins (2001) in UK researched on colouring our foods in the last and next millennium. An established list of permitted synthetic colours eventually came into force in most countries early in this century. In the last twenty years however, consumers have become increasingly aware of the ingredients in their foods and as such they require foods to be as 'natural' as possible. This combined with technological developments has fuelled the increase in the usage of naturally derived colours. Colour suppliers are however constantly striving to improve the technical and physical properties of their colour portfolio, to make the use of colour easier, to improve the stability and to meet customer demands on the functional additives used within colour formulations. This paper will review all colours in terms of recent developments and regulations as well as addressing the question of the future of colours in the next millennium.

Ho-Soo Lim, Jae Chon Choi, Sung-Bong Song, Meehye Kim (2014) in republic of Korea made quantitative determination of carmine in foods by high performance liquid chromatography. Samples were homogenised and extracted with 0.05 M NaOH, followed by centrifugation. The resulting solution was filtered and injected to HPLC. Carmine was separated by HPLC using an NovaPak C_{18} column coupled to a photodiode array detector. The contents of carmine were finally quantified using corresponding calibration curves over ranges of 1.0–100 μg ml^{-1}, with good correlation coefficients ($r^2 = 0.9999$). The recoveries of carmine from foods spiked at levels of 10, 50, and 100 μg g^{-1} which ranged from 90.4% to 96.2% with relative standard deviations between 2.8% and 6.8%. Limit of detection and limit of quantification of carmine were 0.4 and 1.0 μg ml^{-1}, respectively. This method was found to be useful to distinguish carmine from carminic acid, a major component of cochineal extract. The method has been successfully applied to various foods.

CHAPTER 3

AIMS AND OBJECTIVES

AIM

To determine the quantitative and qualitative analysis of food colourant in candy.

OBJECTIVES

To investigate the presence of food colours like brilliant blue, sunset yellow and tartrazine in Poppins and to evaluate the quantity of food colours like brilliant blue, sunset yellow and tartrazine.

CHAPTER 4

MATERIALS AND METHODOLOGIES

APPARATUS REQUIRED :

Woollen thread, Dil. HCL solution, Ammonia, water bath etc..

Beaker, Reagent bottles, standard flask , Filtration units , 0.2 filter, Sonicater etc..

REAGENTS :

Standard solution, working solution.

INSTRUMENTS

HPLC with UV detector

Detector condition:

- Tartrazine – 420nm

Mobile phase : acetonitrile : 0.1 molar ammonium acetate in HPLC Water : 60:40 v/v.

- Brilliant blue – 600nm

Mobile phase : acetonitrile : 0.1 molar ammonium acetate in HPLC water :30:70 v/v.

- Sunset yellow – 495nm

Mobile phase : acetonitrile :0.1 molar ammonium acetate in HPLC water : 70:30 v/v.

- Flow rate : 1 ml/minute flow
- Volume for injection : 20µl
- HPLC column :C 18 (4.6 x 250mm)4µ

METHODS

Reagent preparation:

Preparation of standard solution:

Accurately weigh 20 mg of each dye into 50 ml standard flasks. Dissolve the food colour standards with HPLC water, the dilute to volume with HPLC water. Prepare the required concentration of working standard from the stock solution.

10 mg of standard weighed and made up to 50 ml with HPLC water in to a volumetric flask.

10/50x1000 =200 mg/L

ie. 200ppm

Preparation of working standard:-

Transfer 1 ml of main stock standard to 10 ml standard flask and dilute with methanol water (10:90) to produce the strength of 20 ppm.

200x V1 = 10 ml x 20ppm

V1 = 200/200 = 1ml

1ml of 200 ppm made upto 10 ml with (methanol: HPLC water ::10:90 v/v) to make up the strength of 20 ppm

Preparation of sample:

Weigh about 5.0g of sample. Add 10 ml of methanol: HPLC water (10:90 v/v) to the sample using a measuring cylinder. Sonicate it for 16 minutes. Centrifuge the extract at 4500 rpm for 10 minutes and inject 20µl to the system.

Extraction of the colour from the candy:

20 cm of woollen thread into a beaker containing about 35 ml of the prepared acidified solution of the sample and boiled for a few minutes till the woollen thread is dyed. Take out the woollen thread and wash it with tap water. Transfer the washed woollen thread to a small beaker containing dilute NH3 and heat again. If the colour is stripped by the alkali the presence of an acid synthetic dye is indicated. Remove the woollen thread. Make the liquid slightly acidic and boil with a fresh

piece of woollen thread. Continue boiling until the colour is taken by the woollen thread extract the dye from the woollen thread. Extract the dye from the woollen thread again with a small plug of cotton and concentrate the filtrate over a water bath. This double stripping technique usually gives the colour extract.

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbant material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. The sample mixture to be separated and analyzed is introduced, in a discrete small volume (typically microliters), into the stream of mobile phase percolating through the column. The components of the sample move through the column at different velocities, which are a function of specific physical interactions with the adsorbent (also called stationary phase). The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called its retention time. The retention time measured under particular conditions is an identifying characteristic of a given analyte. [11]

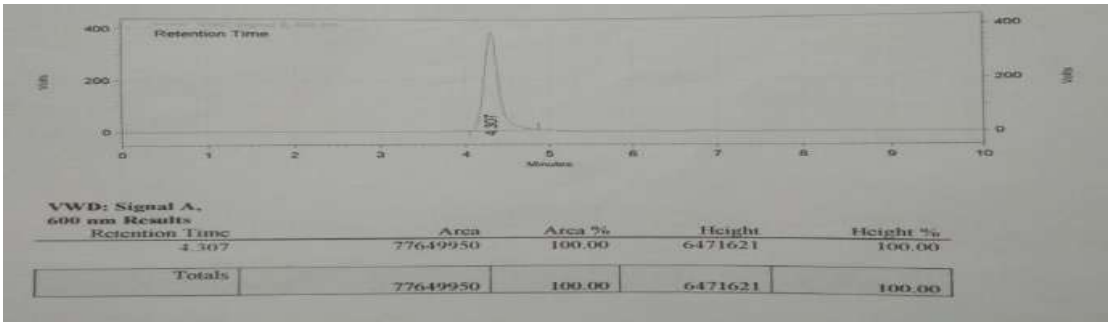
CHAPTER5

CALCULATION AND OBSERVATION

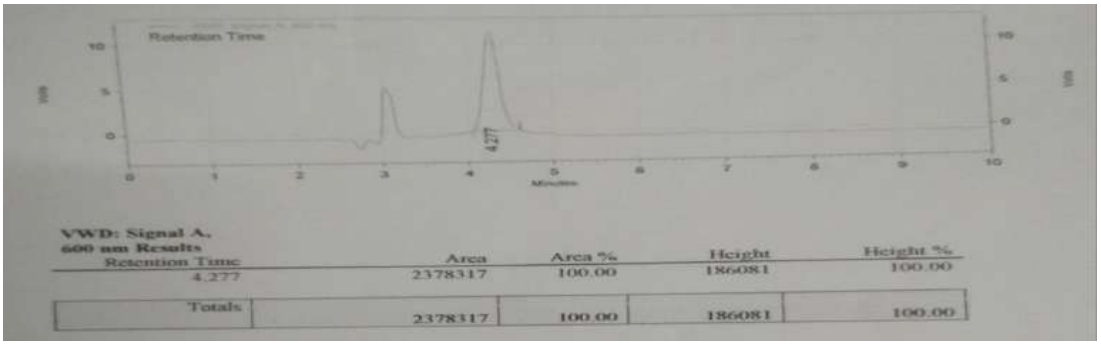
General equation

$$\text{conc of sample} = \frac{(\text{area of sample})\text{x}\ (\text{conc of standard})\text{x}\ (\text{final volume})}{(\text{area of standrad})\ \text{x}\ (\text{weight of sample taken})}$$

- Brilliant blue



Graph1. Graphical representation of % area report of standard of brilliant blue.



Graph. 2 Graphical representation of % area report of sample of brilliant blue.

Observation table no:1

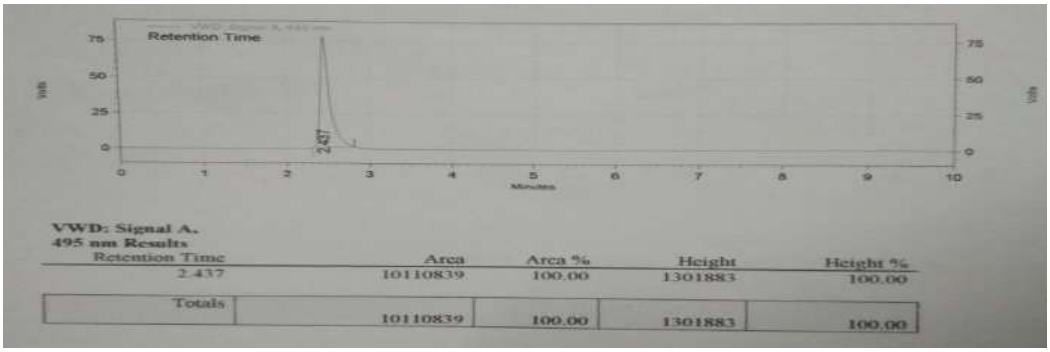
Area of sample	2378317
Area of standard	77649950
Height of sample	7.153g
Conc of standard	20 ppm
Final volume	20 ml

Therefore,

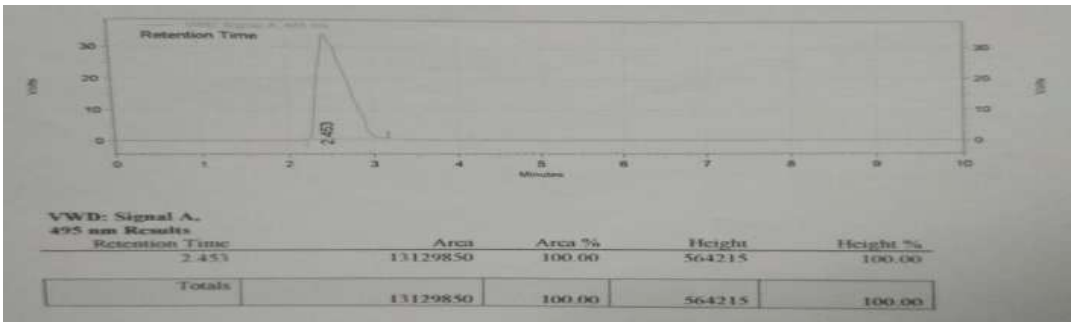
$$\text{Conc of brilliant blue} = \frac{2378317x20x20}{77649950x7.153}$$

$$= 1.71\text{ppm}$$

- Sunset yellow



Graph 3. Graphical representation of % area report of standard of sunset yellow.



Graph 4.graphical representation of % area report of sample of sunset yellow.

Observaton table no:2

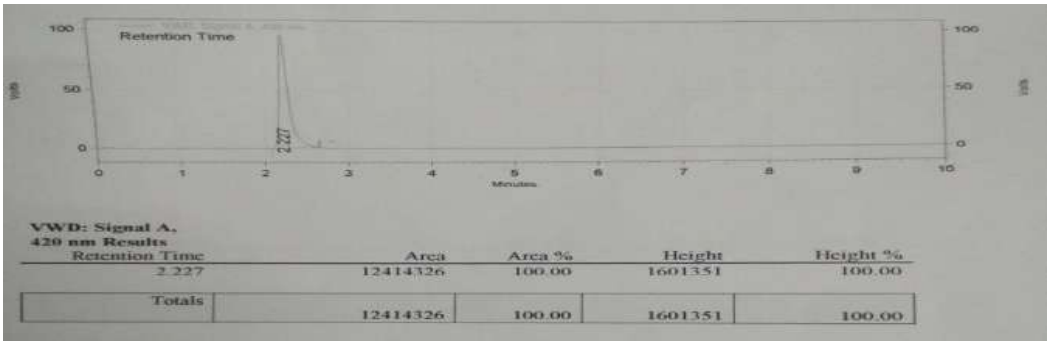
Area of sample	13129850
Area of standard	10110839
Weight of sample	7.9282g
Conc of standard	20ppm
Final volume	20ml

Therefore,

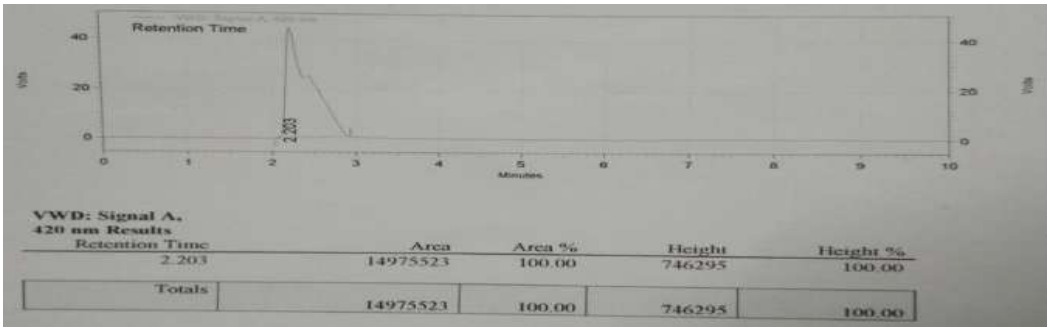
$$\text{Conc of sunset yellow} = \frac{13129850 \times 20 \times 20}{10110839 \times 7.9282}$$

$$= 65.5 \text{ ppm}$$

- Tartrazine



Graph 5.Graphical representation of % area report of standard of tartrazine.



Graph 6. graphical representation of % area report of sample of tartrazine.

Observation table no:3

Area of sample	14975523
Area of standard	12414326
Weight of sample	7.2349g
Conc of standard	20 ppm
Final volume	20 ml

Therefore ,

$$\text{Conc of tartrazine} = \frac{14975523 \times 20 \times 20}{12414326 \times 7.2349}$$

$$= 66.69 \text{ppm}$$

CHAPTER 6

RESULT AND CONCLUSION

RESULT:

In brilliant blue 1.71ppm colourant is detected in poppins brand.In sunset yellow 65.5 ppm colourant is detected in poppins brand.In tartrazine 66.69 ppm colourant is detected in poppins brand.

CONCLUSION:

Comparatively brilliant blue is having lesser quantity of food dye while sunset yellow and tartrazine is having more quantity but within the limit of food safety and standard regulation (FSSR).

CHAPTER 7

REFERENCES

1. Alison Downham and Paul Collins (2001), UK, Colouring our foods in the last and next millennium. Volume 18 pages 643-652.

2. A Hussain, W sawaya, A Al Omair, S. Al Zenkin, H. Al Amiri , N Ahmed and M. Al Sinan (2005), dietary exposure of children to artificial food colours in Kuwait.

3.Meenakshi Tripathi, Subash K. Khanna, Mukul Das (2005), lucknow ,surveilliance on useof synthetic colours in eatables vis a vis prevention of food Adulteration Act of India. Volume 18 pages 211-219.

4. Laura M. konig and britta renner (2007),Meal colour variety and its consumption of food. Volume 64 pages 66- 71.

5.Frank E Lancaster and James F Lawrence(2009) , presence of glucose and sucrose by thermal decomposition of the food colours amaranth, sunset yellow FCF tartrazineMixtures of food colour, sucrose and glucose were heated at several temperatures under simulated candy making. Volume 732 pages 394-398.

6. Wajih Sawaya, Adnan Hussain, Fawsiya Al-Avadhi, Naal Al-Hamad, Basma Dashti, Jameela Al-Saqger(2007) , Kuwait, Consumption pattern of artificially coloured foods among children in Kuwait. Volume 19 pages 98-105.

7. M. Cecilia F.Toledo, Monica S. Guerchon and Sidnei Ragazzi(2009) ,Brazil, Potential weekily intake of artificial food colours by 3- 14-year- old children.

8. Bakthi Petigara harp, Enio Miranda-Bermudez, Carolina I.Baron, and Garald I. Richard (2012), US , Quantitatively tested whether the permited amount of colour additives in food products.

9. Ho-Soo Lim, Jae Chon Choi, Sung-Bong Song, Meehye Kim (2014), republic of Korea, Quantitative determination of carmine in foods by high performance liquid chromatography. Volume 158 pages 521-526.

10. Charles Spence(2015), the physiological impact of food colour. Volume 11 pages. 41-48.

11. https://en.wikipedia.org/wiki/High-performance_liquid_chromatography.

12. https://en.wikipedia.org/wiki/Food_coloring.

13. https://en.wikipedia.org/wiki/Candy.

14. http://www.eatingwell.com/article/16442/the-hidden-health-risks-of-food-dyes/.

15. drugfuture.com

16. sigmaaldrich.com

17. researchgate.net