Original Research Article

Identification of synthetic food colors adulteration by paper chromatography and spectrophotometric methods

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Abstract

Synthetic food colors are widely used in different types of food stuffs in India as well as in the world. Changing lifestyles across the globe have transformed food habit patterns. The instant and processed foods (junk foods) are mainly used in a variety of attractive "Synthetic food colors" by its manufacturers. In the present study an attempt has been aimed to study the Extraction, Identification of Synthetic food colors adulteration by Paper chromatography and UV-Visible spectrophotometer in different sweets and jams. In the Institute of Forensic Science and Criminology (IFSC), Bundelkhand University, Jhansi, Uttar Pradesh in 2011.

Key words

Synthetic food colors, Synthetic food colors classification, Synthetic food color adulteration and toxic effects, Extraction, Paper chromatography, UV-VIS Spectrophotometer.

Introduction

"***Synthetic food colors*** are substances of chemicals which do not occur in nature and have been made in factory". These colors are usually water-soluble and can be used in foods without any further processing [1-3]. This means that they have to be detected or enhanced to be further examined as a part of a standard Forensic procedure. Knowledge of chemical composition and changes over time proved to be an important factor in the development of new detection techniques for synthetic food colors [4-7]. Examples of synthetic food colors: Ponceau 4R, Carmoisine, Erythrosine, Tartrazine, Sunset Yellow FCF [8, 9].

Characteristics

- Color is uniform
- Highly stable to light, oxygen and PH.
- Inexpensive
- Harmful to humans if exceed 0.2 g/kg of food [10-12].

Classification

Classification of Synthetic food colors are mentioned in the following **Table – 1**. The first synthetic dye is Mauveine, from which oxidizing aniline developed by Sir William Henry Perkin (1856). At the end of the century, the principle sources of synthesized dyes are eighty synthetic dyes colored foods, and coal tar derivatives. According to the importance of Synthetic food colors a number of methods for their extraction and identification have been developed. Extraction from the food sample is carried out either by adsorption on wool. Many methods have been used for the identification of the different colors used in food processing. In 1860, Hassall passed the first Food Adulteration Act; he analyzed the food samples firstly with a Microscope for identifying foreign organic matter, for which no chemical tests were available. Arata (1889) developed the use of wool fibers for extraction and cleanup of food samples for colorant analysis [13-17].

Table - 1: Synthetic food colors are classified as per prevention of food adulteration (PFA) act.

SI. No.	Color	Common Name	Color Shade	Color Index	Chemical Class	Empirical Formula
1.	Red	Ponceau 4R	Strawberry Red	16255	Mono azo	$C_{20}H_{11}N_2O_{10}S_3Na_3$
		Carmoisine	Red	14720	Mono azo	$C_{20}H_{12}N_2O_7S_2Na_2$
		Erythrosine	Bright Pink / Red	45430	Xanthene	$C_{20}H_8O_5I_4Na_2$
2.	Yellow	Tartrazine	Lemon Yellow	19140	Mono azo	$C_{16}H_9N_4O_9S_2Na_3$
		Sunset Yellow	Orange	15985	Mono azo	$C_{16}H_{10}N_2O_7S_2Na_2$
3.	Blue	Indigotine carmine	Royal blue	73015	Indigoid	$C_{16}H_8N_2O_8S_2Na_2$
		Brilliant Blue FCF	Turquoise Blue	42090	Triarylmethane	$C_{37}H_{34}N_2O_9S_3Na_2$
4.	Green	Fast Green FCF	Sea Green	42053	Triarylmethane	$C_{37}H_{34}N_2O_{10}S_3Na_2$

Traditionally chromatographic methods are used. Paper chromatography plays the most important role. The applicability of the method was tested with a variety of synthetic food colorant sweets. It was found that the results are reliable, obtained by Paper chromatography [18-20]. Ronald L. Stanley and Paul L. Kirk (1963) developed systematic identification of artificial food colors which are permitted in the United States. They are analyzed about artificial dyes in food and using Wool dyeing for screening, column adsorption on alumina for isolation and paper chromatography for isolation and identification [30]. David A. Katz (2009) developed the Extraction, separation and identification of synthetic food colors from various foods like candies and soft drinks [9]. The most important method for the identification of synthetic food colors is UV Visible Spectroscopy, because all colorants have been characterized by this method. Sedat Sayar and Yaksel Ozdemir (1996) developed derivative spectrophotometric methods. These were originally developed for deterring Ponceau 4R and Tartrazine in order to resolve their binary mixtures. It permits the simultaneous determination of these food colorants in various food samples [32].

Synthetic food color adulteration

Synthetic food colors serves to mask defects in food making and inferior foods look superior. Synthetic food colors react with food and change into poisons in the body, causing mutations, cancer and other toxic effects. These colors

produce allergic reactions in several individuals [21, 22].

Causes of Synthetic Food Colour Adulteration

- Consumers demand color and variety in foods.
- The traders make their goods look superior, attractive and thereby increase of sales and profit.
- Consumer ignorance, carelessness, indifference and lack of organized action of check the menace.
- Inadequate enforcement of foods and absence of deterrent punishment for offenders [23-25].

Toxic Effects of Synthetic Food Color on Human Health (Photo – 1)

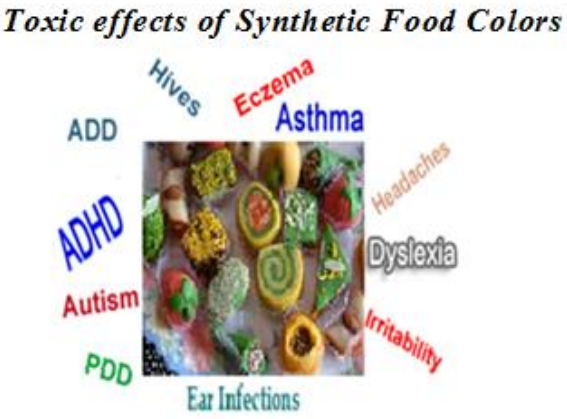
- They are carcinogenic and damage to kidneys and adrenals.
- Synthetic food colors, lowers the red cell count and hemoglobin concentration and allergic reactions.
- They inhibits dopamine uptake by nerve ending (reduced dopamine turnover).
- These colors are associated with irritability, restlessness and sleep disturbance in atopic or hypertensive more effective to children than adults, effects on liver and Intestine.
- They cause exhibited symptoms glossitis (Inflation of tongue). Allergic responses vary from uticaria to dermatitis, angioedema, etc.
- They lead to Ear infections, Asthma, Dyslexia, Eczema, Autism, etc. when consumption of high level of synthetic food colours [26-35].

Materials and methods

Apparatus and Chemicals

Whatman filter paper No. 31ET, Sodium acetate, Ammonia, distilled water, filter paper, Paper chromatography chamber. Schimadzu UV-VIS Spectrophotometer with 1.0 cm quartz cell was used for the measurement of absorbance synthetic food colors.

Photo – 1: Toxic effects of synthetic food colors.



Standards

Synthetic food colors were purchased from Jhansi. These colors are manufactured by Arun color chemicals and Ajanta Industries, Gurgaon, Haryana. These colors are certified by ISO 9001:2000.

Preparation of Standard Stock Solutions

Individual solutions were prepared at 1g / 1000 ml with distilled water. 0.5ml of solution was taken in another test tube and volume was made up to 50ml to make stock solution. The prepared stock solution stored at room temperature. A series of standard solutions were freshly prepared during analysis.

Samples

In the present work initially, a market survey was carried out to identify various colourful sweets and the sweets were purchased from shops, sweet stalls, street vendors in Jhansi city of Uttar Pradesh, India 2011. All samples are collected from Elite Chouraha, Sadar bazaar, Shehar, Sipri bazaar at Jhansi in Uttar Pradesh which is mentioned in the following **Table - 2**.

Preparation of Sample solutions / Extraction Method

Common Arata Method using Water Bath (or) Add small quantity (2 g) of sweet sample was measured and dissolved in 10ml of distilled water in a test tube. Then place it in centrifuge at 50,000 rpm. After 10 to 15 minutes a test tube is taken out from the Centrifuge. Then, the sample

solution was filtered. The filtrate was then collected. 0.5 ml of filtrate was taken in another test tube and volume was made up to 3ml by adding with distilled water. Preserve solution in a stopper glass bottle for further analysis. This process is same for all the sweet samples.

Color Tests

Color tests are based on colour change reactions in the presence of specific reagents such as conc. HCl, Conc. H_2SO_4, NaOH, NH_3. The colour change reactions of synthetic food colorants in the presence of specific reagents in the following **Table – 3**.

Table - 2: The collected sweet samples and its Synthetic food color. (**Photo – 2**)

Name of the sweet	Color of the sweet
Kaju pista leechi	Pink
Kaju pista leechi	Light green
Burfi	Dark Green
Pista Burfi	Parrot Green
Malai chap	Dark orange
Moti choor Laddoo	Light orange
Burfi	Light Brown
Burfi	Dark Brown
Boondi Laddoo	Yellow
Jam	Red

Table - 3: Synthetic food colors preliminary examination through color Tests in presence of reagents.

SI. no.	Name of The Color		Conc. HCl	Conc. H_2SO_4	NaOH	30% NH_3
1.	Pink	Standard	Very Light pink	Dark Pink	Light Pink	Dark Pink
		Sample	Very Light pink	Dark Pink	Light Pink	Dark Pink
2.	Light Green	Standard	Very Light yellow	Light yellow	Light green	Very light green
		Sample	Very Light yellow	Light yellow	Light green	Very light green
3.	Dark green	Standard	Light yellow	Dark yellow	Light green	Very Dark green
		Sample	Light yellow	Dark yellow	Light green	Very Dark green
4.	Parrot green	Standard	Very light yellow	Dark yellow	Light green	Slightly dark
		Sample	Very light yellow	Dark yellow	Light green	Slightly dark
5.	Dark orange	Standard	Slightly dark	Red	Very dark orange	Dark red
		Sample	Slightly dark	Red	Very dark orange	Dark red
6.	Light orange	Standard	Orange	Very light orange	Dark orange	Very dark orange
		Sample	Orange	Very light orange	Dark orange	Very dark orange
7.	Light brown	Standard	Very light red	Dark pink	Pink	Pink
		Sample	Very light red	Dark pink	Pink	Pink
8.	Dark Brown	Standard	Light red	Red	Dark pink	Very dark brown
		Sample	Light red	Red	Dark pink	Very dark brown
9.	Yellow	Standard	light yellow	very dark yellow	Light yellow	Light yellow
		Sample	light yellow	very dark yellow	Light yellow	Light yellow
10.	Red	Standard	Light red	Dark violet	No change	Dark red
		Sample	Light red	Dark violet	No change	Dark red

Photo – 2: Synthetic food color sweet.

Paper chromatography

Principle of Paper Chromatography

Paper is a type of partition chromatography in which compounds are distributed between 2 liquids (Stationary phase and Mobile phase). Stationary phase is the fibres of the paper. Mobile phase is liquid or developing solvent.

Choice of paper: Whatman filter paper No. 31ET is used for paper chromatography.

Solvent: Sodium acetate (3gm) : Ammonia (10 ml): distilled water (90 ml)

Ultraviolet-visible (UV- VIS) spectrophotometer: Principle of UV-visible Spectrophotometer is mainly based on the beer's law and lamberts law

Beer's law: states that the amount of light absorbed by a material is proportional to the concentration of absorbing material. **A α C**

Lambert's law: states that the amount of light absorbed by a material is proportional to the thickness of absorbing material. **A α L**

Schimadzu 1800 UV/ VIS Spectrophotometer was used in this study, the UV/ visible spectrum of a substance over a certain wavelength range consists of absorption values measured at a number of wavelength points.

Results

There is a great need to create awareness at different levels about the toxic effects of synthetic food colors. The present study reported that these synthetic food colors are extracted, and identified by paper chromatography and UV-Visible Spectrophotometer. Mainly to detect the presence of synthetic food colors adulteration in sweets (Kaju Pista Leechi, Burfi, Moti choor ka Laddoo, Boondi ka Laddoo) and jam.

In paper chromatography, by using solvent {sodium acetate (3 g): ammonia (10 ml): distilled water (100 ml)}, samples and standards are compared with the Rf value. Based on the Rf value of standard and samples, these synthetic food colors are separated and identified. Paper chromatographic technique is shown that 2 (Parrot green and light brown) out of 10 sweet samples are found to be adulterated. Rf values are mentioned as per **Table - 4** and Some developed Paper Chromatograms were as per **Figure - 1**.

In UV-Visible spectrophotometer, the absorption of Synthetic food colors in both standards and samples were compared. It is found that 9 out of 10 sweet samples are found to be adulterated, it shown that dark orange color sweet is not adulterated by synthetic food color. Absorbance of standards and samples were mentioned as per **Table - 5**.

Absorbance of Standards and Samples are graphically represented at particular wavelength as per **Figure - 2**. Statistical data representation of Standard and Sample was as per **Table – 6**.

Graphical representation of correlation between standard and sample was as per **Figure – 3**.

Table - 4: Rf values of samples and standards.

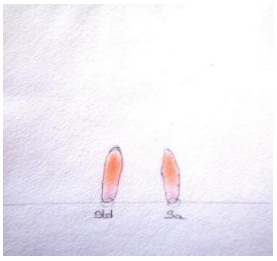
Synthetic food Color	Rf value of standard	Rf value of sample
Pink	0.16	0.14
Parrot green	0.58	0.49
Light green	0.25	0.24
Dark Green	0.56	0.54
Dark orange	0.52	0.50
Light orange	0.21	0.19
Dark brown	0.22	0.21
Light Brown	0.45	0.41
Red	0.27	0.26
Yellow	0.25	0.24

Figure - 1: Developed paper chromatograms.

Pink

Light orange

Light brown



Red

Discussion

In the present work, 10 Synthetic food colors from 10 different types of sweet samples (Kaju Pista Leechi, Burfi, Moti choor ka Laddoo, Boondi ka Laddoo, Jam), which are collected in Jhansi city of Uttar Pradesh. The Synthetic food colors are extracted from sweet samples are analyzed by Paper Chromatography the synthetic food colors are separated and identified based on the Rf value and identified the Rf value of the synthetic food colors of standards and samples, followed by the findings of David A. Katz (2009) who has also used Paper Chromatography and experimented on only five synthetic colors such as Blue 1, Blue 2, Yellow 5, yellow 6, Red 3. So, the results were similar to David A. Katz [9].

Sedat Sayar and Yuksel Ozdemir (1996) experimented on Ponceau 4R and Tartrazine Synthetic food color in binary mixture in different samples by derivative spectrophotometric method [32]. They compared the absorbance of standard and samples absorbance of 2 synthetic food colors, graphically represented and then statistical represented. In present work, the standards and samples are identified by UV-Visible Spectrophotometer. Based on the wavelength of standards and samples absorption compared and graphically represented and statistically representation of correlation by S.P.S.S. Software and Regression was calculated. The result of the present study shows similarity with Sedat Sayar and Yuksel Ozdemir [32].

Table - 5: Absorbance of the standards and samples.

Synthetic food color	Solvent	Wavelength (nm)	Blank Absorbance	Standard Absorbance	Sample Absorbance
Pink	Distilled Water	510 nm	0.003	1.00	2.88
Parrot green	Distilled Water	628 nm	0.17	0.48	0.20
Light green	Distilled Water	628 nm	0.17	1.77	0.82
Dark green	Distilled Water	628 nm	0.17	1.80	0.94
Dark orange	Distilled Water	480 nm	0.007	4.00	4.00
Light orange	Distilled Water	482 nm	0.007	2.67	0.58
Dark brown	Distilled Water	520 nm	0.004	1.65	0.83
Light Brown	Distilled Water	520 nm	0.004	2.55	1.77
Red	Distilled Water	510 nm	0.003	4.00	2.61
Yellow	Distilled Water	425 nm	0.019	3.73	2.12

Table - 6: Statistical data representation of Standard and Sample.

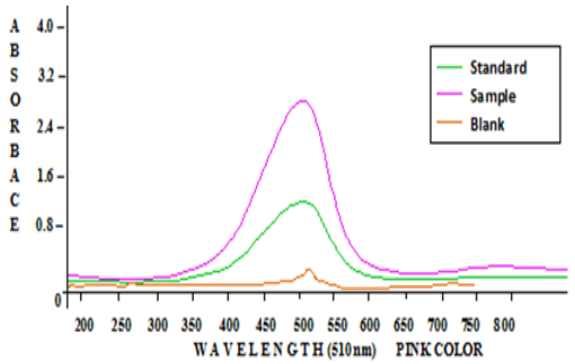
Absorbance	Mean value	Correlation value "r" , (n = 10)	Regression equations
Standard (X)	2.365	0.609*	X = 0.803 Y +0.699
Sample (Y)	1.675		Y = 0.852 X – 0.057

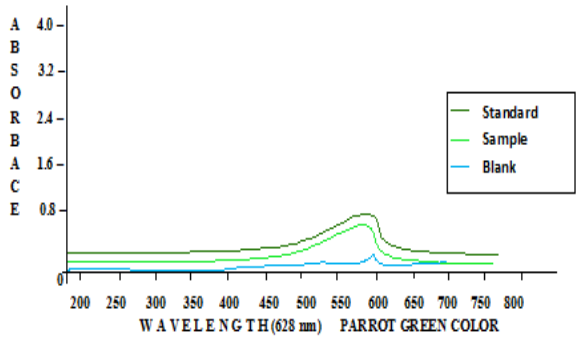
*1% level of significance

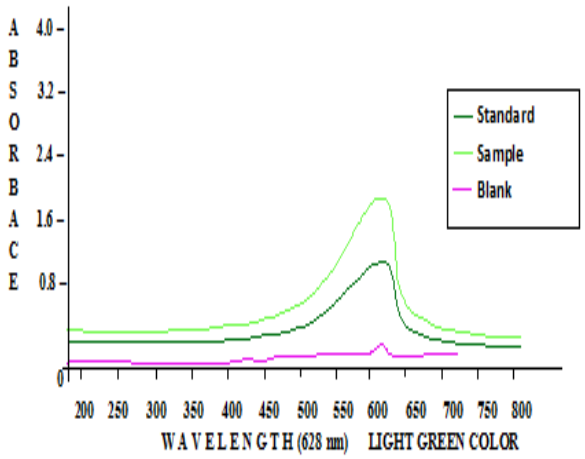
Conclusion

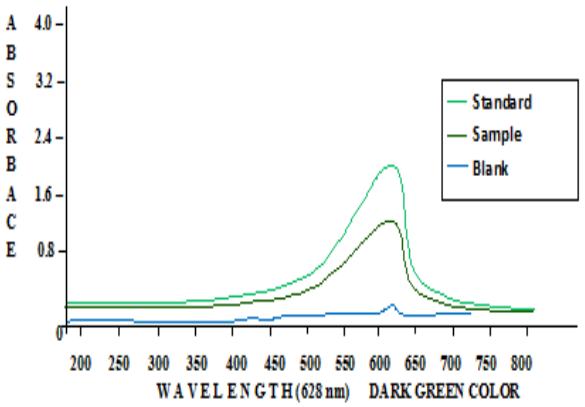
It is concluded that color tests and paper chromatography is preliminary examination for synthetic food colors. UV-Vis spectrophotometer is widely used for qualitative analysis and the data presented in various tables and graphs show that the remarkable identification of adulteration of synthetic food colors. This study concluded that the techniques and data can be used for adulteration identification in different types of food stuffs. Nowadays, synthetic food colors are frequently used, therefore results of the study is found to be very useful to prevent the malpractice of synthetic food color adulteration. The study is found to be fruitful for Forensic Chemistry and Toxicology. It has to be assessed very carefully because of the Medico-legal importance. Adulterations may lead to food poisoning. Synthetic food color adulterations are more vulnerable even to lesser toxicity and food poisoning and sometimes death may also occur especially in children.

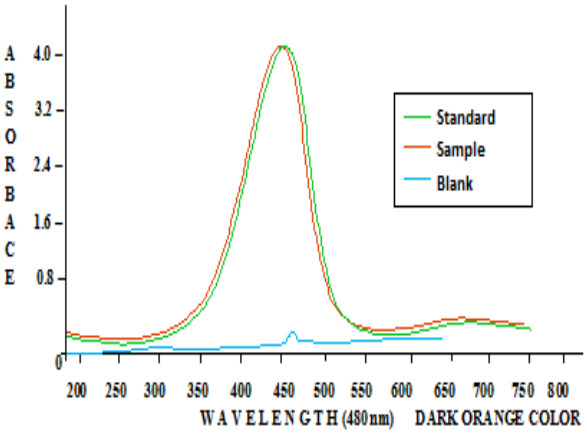
Figure - 2: Graphical Representation of Blank, Standards and Samples absorbance by UV-Visible Spectrophotometer.

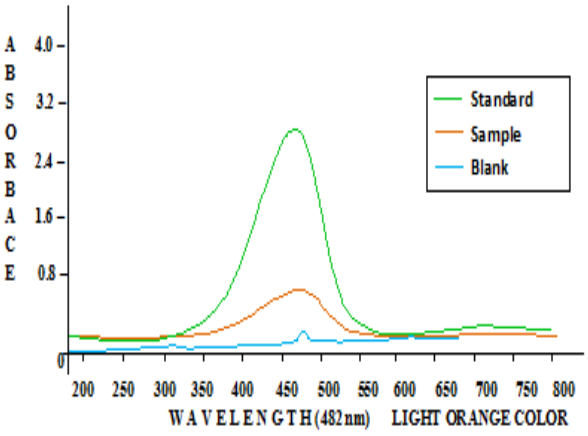












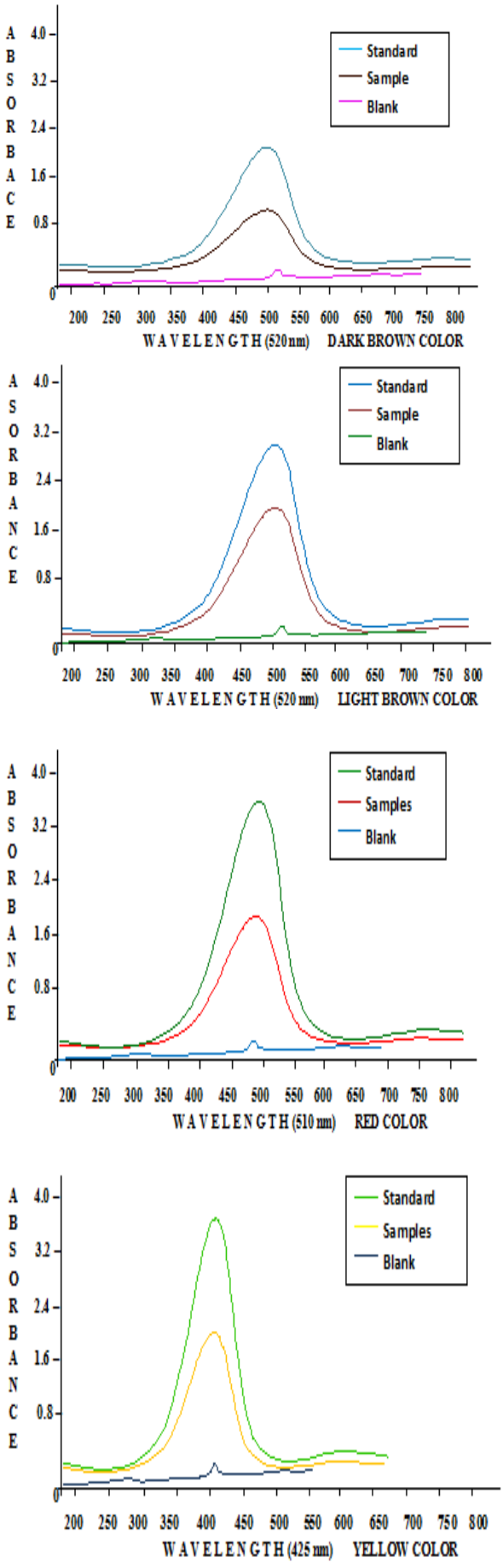
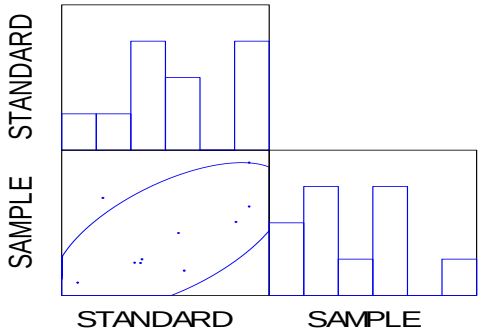


Figure – 3: Graphical representation of correlation between standard and sample.



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