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ESTIMATION OF TARTRAZINE IN FRUIT JUICES AND JAMS BY HPTLC METHOD DEVELOPMENT AND VALIDATION PARAMETERS

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ABSTRACT: Tartrazine is a lemon-yellow synthetic food colourant belonging to azo group. The Acceptable Daily Intake (ADI) of tartrazine is 7.5mg/kg However, it is possible that more than permissible amount of Tartrazine is used to make food items more attractive. This may pose threat to consumer's health. Hence the overall aim of the study was to detect and quantitate the presence of tartrazine in commercially available as well as locally made and sold fruit juices and jams. Fresh fruit juices (Branded and Non-Branded) and jams purchased from shops and street vendors in Belagavi city of Karnataka, India were subjected to High Performance Thin Layer Chromatography (HPTLC). Silica gel aluminum plate $^{60}F_{254}$ was used as the stationary phase and Iso-propanol: Ammonia (6:4) v/v was used as mobile phase. HPTLC has many advantages over the other analytical methods applied for tartrazine estimation. The method was validated using analytical parameters like linearity, range, precision, specificity, LOD, LOQ and robustness. Out of the 10 branded fruit juices analysed tartrazine was present in 9 samples within permissible range. Tartrazine was absent in local juices. Tartrazine was present in all 10 jam samples analysed within permissible range. Presence of tartrazine within ADI range suggest that the juices and jams are safe for consumer's health.

INTRODUCTION: Visual appearance is one of the important attributes of the food materials. The appearance of the dish can either stimulate or dampen appetite and may lead to happiness or utter dejection [1]. Since, natural pigments are more expensive and easily decomposed, they are less frequently used in the food industry even though they are safe for consumption [2]. The benefits of synthetic food colours over natural food colours include affordability, colour stability and resistance to pH changes [3]. The greatest consumers of synthetic food colours are children, who are more drawn to colourful meal plates.

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Over the past 50 years, there has been a 500% increase in food colour consumption by children [4]. Over 70% of all commercially used dyes are azo dyes [5]. Azo dyes are categorised as aromatic compounds having (R-N=N-R'), where R and R' can be alkyl or aryl [6]. Tartrazine (E 102) is a water-soluble azo dye that gives lemon-yellow colour to foods, beverages, and pharmaceuticals. Tartrazine is mostly utilized in foods such as carbonated drinks, soups, chips, cotton candies, juices, pastries, and jams [7].

In certain nations, tartrazine is illegally used as an alternative to saffron [8]. Several studies done on humans have demonstrated adverse effects of tartrazine. According to studies done on rats, tartrazine is toxic, impairing haematological and neurobehavioral function, as well as producing hyperactivity, anxiety, depression, and antisocial behavior [9, 10].

According to studies done on humans, tartrazine is known to cause urticaria, hyperactivity in children, and effects on DNA repair in human lymphocytes [11, 12, 13]. Considering the adverse impact of tartrazine on health, the Joint FAO/WHO Expert Committee on Food Additives [JECFA] and EU Scientific Committee for Food (SCF) has prescribed the Acceptable Daily Intake (ADI) for tartrazine 7.5 mg/kg body weight [14]. The Food Safety and Standard Authority of India (FSSAI) has established 100 ppm as the highest allowable concentration for tartrazine in the finished food product [15]. Prior to the European Parliament and Council Directive 94/36/EC lifting the restriction, tartrazine use was prohibited in Austria, Germany, and Norway [16].

In this study, we report the presence and levels of tartrazine in juices and jams that are commonly consumed.

MATERIALS AND METHODS:

Samples: Fruit juices (10 branded and 10 non-branded) and 10 jams were purchased from shops and street vendors in Belagavi city of Karnataka, India.

Preparation of Tartrazine Stock Solution: The dye tartrazine used in the study was purchased from Himedia Laboratories, India- Catalog number (A17682.36). 10 ml of stock solution of tartrazine in Millipore water at 100 µg/ml was prepared as the standard.

Method Development:

Determination of λmax: UV-visible spectrophotometer was used to scan a solution containing 10 µg/ml of tartrazine to determine λmax of tartrazine. Millipore water was used as blank.

HPTLC Method Validation: The developed HPTLC method was then validated according to International Council for Harmonisation (ICH) guidelines. Various parameters used for validation were linearity, specificity, precision, LOD (Limit of Detection), LOQ (Limit of Quantification) and robustness

Linearity: To test linearity standard tartrazine solutions with a concentration of 0.2 to 1µg/ml were prepared from stock solution and evaluated

Precision: Six replicates of the stock solution of 100µg/ml concentration were studied to test precision of developed analytical approach.

Specificity: In order to assess specificity of the approach, the standard stock solution was put through three different concentrations being low (0.2µg/ml), medium (0.6µg/ml) and high (1.0µg/ml).

LOD and LOQ: The least amount of analyte that may be identified in a sample is LOD. Least amount of sample that can be precisely and accurately quantified is LOQ. LOD and LOQ of the analyte were calculated using following formulae.

$$LOD = 10 \times (s/m) \text{ and } LOQ = 3 \times (s/m)$$

Where, m is the slope of the associated calibration curve s is the standard deviation response.

Robustness: The robustness of the approach was evaluated using 100µg/ml solutions of tartrazine at 3 different mobile phase ratios (6:4, 7:3 and 8:2 v/v)

Sample Preparation:

Fruit Juices: 160 ml of fruit juices were heated by placing on water bath at 90°C to evaporate to half of its water content.

Jams: 10 mg of jams were precisely weighed and added to eppendorf tube filled with 1 ml Millipore water. The jam was thoroughly mixed with the water. The eppendorf tube was then centrifuged at 8000rpm for 5 minutes and the supernatant was transferred into fresh tubes for HPTLC analysis.

HPTLC Analysis of the Samples: The stationary phase for the separation of tartrazine was silica gel aluminum plate $^{60}F_{254}$. Using a 100 µl micro-syringe, precise amounts of 5 µl of standard and samples were applied on base of the plate. The chromatographic plate was then put in chamber that was saturated with mobile phase vapors after application of the standard and samples. Optimization of mobile phase was done after trial tests that produced optimum separation of tartrazine. The mobile phase used for the study was Isopropanol: 25% Ammonia (7:3 v/v). The plates were then dried manually using a drier. The distance between the standard and sample were

measured using the software CAMAG vision CATS.

RESULTS:

Method Development:

Wavelength Determination: To determine the maximum tartrazine wavelength, the standard tartrazine solution was subjected to UV-Visible spectrophotometry within the range of 200-800 nm. One peak of tartrazine was obtained in the UV region, whereas the other was found in the visible region. The λ max for tartrazine in the UV and visible region was at 256nm and 426nm respectively **Fig. 1.**

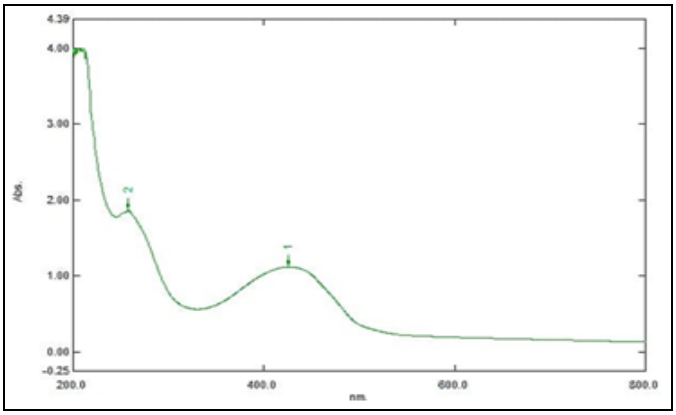

FIG. 1: ΛMAX OF TARTRAZINE

1. 427nm

2. 256nm

Method Validation:

Linearity: With a concentration range of 0.2 to 1.0 µg/ml, a calibration curve for concentrations vs. peak area was developed to test linearity. The correlation coefficients' (R^2) value was found to be 0.9959 **Fig. 2** indicating excellent linearity over the range tested.

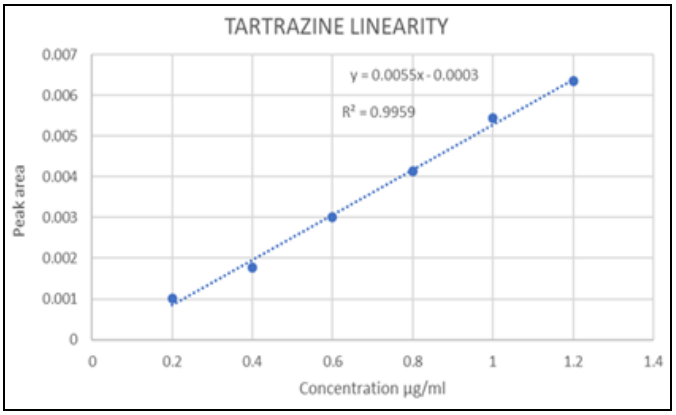

FIG. 2: LINEARITY GRAPH OF TARTRAZINE

Specificity: The method's specificity was established by addition of standard solution in low (0.2 µg/ml), medium (0.6 µg/ml) and high (1.0 µg/ml) concentrations in triplicates. At all three concentrations %RSD value was below 2 **Table 1.**

TABLE 1: SPECIFICITY RESULTS

Level	Concentration (µg/ml)	Volume (µl)	Rf	Peak area	Mean	SD	%RSD
Low	0.2	5	0.64	3068.3	3006.33		
	0.2	5	0.63	2992.5		56.34	1.87
	0.2	5	0.64	2958.2			
Medium	0.6	5	0.63	4467.1			
	0.6	5	0.64	4367.7	4443.93	67.69	1.52
	0.6	5	0.64	4497			
High	1.0	5	0.64	5699			
	1.0	5	0.63	5714.6	5658.23	84.48	1.49
	1.0	5	0.64	5561.1			

Precision: Precision study was performed by applying 6 replicates of 100 µg/ml of tartrazine stock solution. The Rf value for all six replicates was 0.63 and % RSD for the peak area was 1.82 **Table 2.**

TABLE 2: PRECISION RESULTS

Concentration (µg/ml)	Rf	Peak Area	Statistical Analysis
100	0.63	4267.8	Mean = 4304.5
100	0.64	4197.3	
100	0.63	4310.3	SD = 78.16
100	0.63	4264.7	
100	0.63	4405	%RSD = 1.82
100	0.63	4381.9	

LOD (Limit of Detection) and LOQ (Limit of Quantification): The LOD of tartrazine was 0.20µg/ml and LOQ was 0.62 µg/ml.

Robustness: Study's robustness was assessed by modifying mobile phase ratio at 6:4, 7:3 and 8:2. Absence of significant change in Rf values in all 3 mobile phase ratios (Rf range: 0.63 to 0.65) indicates that the analytical method developed is robust **Table 3.**

TABLE 3: ROBUSTNESS RESULTS

Concentration (100µg/ml)	Volume (µl)	Mobile phase ratio	Rf
100	5	6:4	0.65
100	5	6:4	0.64
100	5	6:4	0.63
100	5	7:3	0.63
100	5	7:3	0.63
100	5	7:3	0.63
100	5	8:2	0.63
100	5	8:2	0.63
100	5	8:2	0.63

Fingerprint Analysis of Branded Fruit Juices: Amount of tartrazine in branded fruit juices was analyzed by applying 5µl of standard and samples on silica plate. Out of the 10 branded fruit juices analyzed tartrazine was found to be present in 9 juices. The amount of tartrazine in branded fruit juices ranged between 70.13ng/ml to 200ng/ml. Findings of fingerprint analysis of tartrazine are given in **Table 4.**

TABLE 4: ESTIMATED LEVELS OF TARTRAZINE IN BRANDED FRUIT JUICES

Sr. no.	Branded juices	Concentration
1	Rasna Orange	145ng/ml
2	Rasna Mango	70.392ng/ml
3	Tang Orange	72.4g/ml
4	Tang Mango	73.6ng/ml
5	Frustar Mango	70.13ng/ml
6	Yeah Mango	200ng/ml
7	Cocojal Mango	75.82ng/ml
8	Alo fruit Kiwi	72.4ng/ml
9	Alo fruit Mosambi	70.30ng/ml
10	Roohafza	Not detected

Fingerprint Analysis of Tartrazine in Fresh Fruit Juices: Tartrazine in local fruit juices was estimated by applying 5µl each of standard and samples on TLC plate. Tartrazine was absent in all the 10 juices analyzed.

Fingerprint Analysis of Tartrazine in Jams: Tartrazine in jams was analyzed by applying 5µl each of standard and jam sample on TLC plate. Tartrazine was present in all 10 jam samples and its concentration was estimated. The amount of tartrazine estimated is given in **Table 5**. Tartrazine in jams was present in the range of 59.622 – 1600 ng per 10 mg.

TABLE 5: ESTIMATED LEVELS OF TARTRAZINE IN JAMS

Sr. no.	Jams	Concentration (per 10 mg jam)
1	Mala Pineapple	816.2 ng
2	Mala Mango	59.622 ng
3	Snac Tac Pineapple	627.93 ng
4	Agri Club Pineapple	760.49 ng
5	Agri Club Mango	1140 ng
6	Agri Club Orange	1600 ng
7	Manama Pineapple	652.51 ng
8	Kwik Snack Mango	581 ng
9	Chellam Pineapple	555.34 ng
10	Patanjali Pineapple	640.59 ng

DISCUSSION: The Acceptable Daily Intake of tartrazine is 7.5mg/kg. Considering average weight of adults in India as 60 kgs, tartrazine intake should not exceed 450 mg.

Given that an average person drinks 250 ml of fruit juice per day, tartrazine levels were lower than 450 mg, ranging from 0.0176 mg to 0.05 mg in branded fruit juices. The levels of tartrazine for 250g of jam ranged between 1.49mg to 40mg.

Thus, in all food products tartrazine levels were below ADI range making it safe for human consumption. Tripathi *et al.*, (2005) also conducted a similar study to determine the type and quantity of artificial food colouring agents including tartrazine applied to a variety of foods in Lucknow's urban and rural districts.

In samples of crushed ice from urban areas, tartrazine was found to be 20 times higher than the permitted limit, whilst in rural areas, it was found to be 16 times higher [15]. Ihediohanma *et al.* (2014) used spectrophotometric technique to determine the tartrazine levels in locally manufactured plantain chips and the results revealed that eight (32%) of the samples contained more tartrazine than the ADI [17]. A spectophoto-metric study was also conducted by Lawal *et al.,* (2020) in Nigeria, and the results showed that the amounts of tartrazine in two out of five beverages were higher than allowed [18].

Tartrazine levels in milk-based confections were found to be higher than allowed, with a mean of 517 mg/kg conc according to UV-VIS data in a study by Siddhartha *et al.,* (2022) [19]. The quantities of tartrazine and sunset yellow in soft drink samples were all below the recommended upper limit value of 100 g/mL, according to Agbokponto *et al.,* (2022) estimate of tartrazine in soft drinks [20]. Radu Rusu *et al.,* (2020) measured the amount of tartrazine in sodas and snacks, and the results of the study revealed that the intake values did not exceed the permissible limit [21]. Similarly tartrazine levels were lower than ADI according the study done by Long *et al* (2019) in Vietnam [22].

In this study, we measured levels of tartrazine in 10 branded fruit juices, 10 local fruit juices and 10 jams sold in the market using HPTLC. HPTLC has a number of benefits over other analytical techniques. HPTLC can be used for identifying, determining, and separating components from the mixture, in contrast to UV/Vis spectroscopy, which cannot be used for separation purposes. Because HPTLC uses less solvent and requires less sample preparation than HPLC, analysis is less expensive and takes less time. Additionally, this method is straightforward, user-friendly and can be used for analysis of various kinds of coloured and colourless compounds. Very few studies in the literature have applied HPTLC for the measurement of tartrazine in food samples. We found the method robust, specific and sensitive. Nine out of ten branded fruit juices contained tartrazine within permissible limit while one did not contain tartrazine. Tartrazine was not detectable in fresh fruit juices. Although all jams contained tartrazine, it was within permissible limit in all samples. This is indicative of excellent compliance to the regulation related to food dyes.

CONCLUSION: In the present study, HPTLC method was developed and validated for estimation of tartrazine in juices and jams. This method is the best option for quick quantitative evaluation of various samples due to low cost of materials, instrumentation and little scanning time. The estimated values of tartrazine in juices and jams were within permissible limit. Presence of tartrazine in juices and jams within permissible limit suggest that they are safe for consumer's health.

CONFLICT OF INTEREST: The authors have no conflicts of interest regarding this investigation**.**

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