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Techniques for Detection and Measurement of Tartrazine in Food Products 2008 To 2022: A Review

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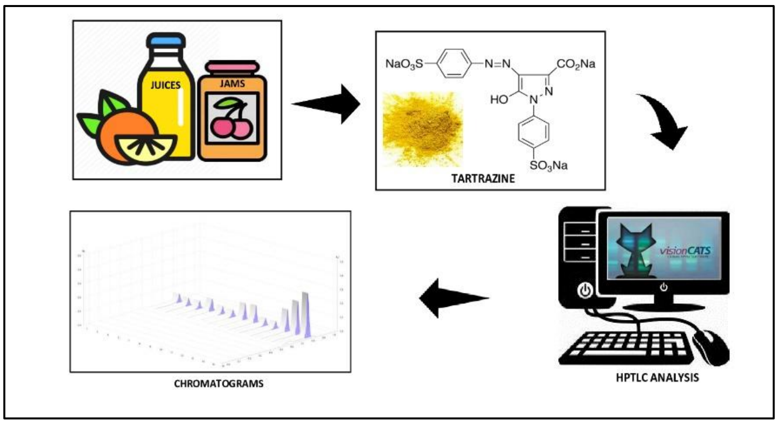
ABSTRACT:

Tartrazine is one of the most commonly used food colourants. It is a synthetic lemon-yellow dye belonging to the azo group. The Acceptable Daily Intake of tartrazine according to the Joint FAO/WHO Expert Committee on Food Additives [JECFA] and the EU Scientific Committee for Food (SCF) is 7.5mg/kg/body weight. However, excessive usage of tartrazine in humans has been shown to have a number of adverse health effects. The detection and estimation of tartrazine is of paramount importance due to its potential impact on consumer health and safety. Chromatographic techniques, such as high-performance liquid chromatography (HPLC) Thin Layer Chromatography (TLC), High-Performance Thin Layer Chromatography (HPTLC), have emerged as robust tools for tartrazine detection. Furthermore, recent advancements in hyphenated techniques HPLC-MS, enhance detection sensitivity and specificity. This abstract outlines the methodologies involving chromatographic and spectroscopic techniques for the sensitive and accurate analysis of tartrazine in various consumables. Chromatographic and spectroscopic techniques offer diverse and complementary approaches for accurate tartrazine analysis, catering to the increasing demand for regulatory compliance and consumer well-being. As technology continues to evolve, these methods are poised to play an essential role in maintaining the integrity of food and beverage products.

Graphical abstract



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Introduction

One of the crucial characteristics of food products is their visual appeal. The appearance of the dish directly influences customer acceptance and choice of food. Colourants/dyes are added to food products all over the world to make it attractive and appealing to consumers. Use of food colours from natural resources are ideal for this purpose. However, they are typically unstable and colour consistency decreases during food processing and manufacturing. As a result, synthetic colourants were developed and are today used in the production of food.

Food colourants are frequently used to enhance the sensory and aesthetic quality of foods because they have stability, excellent colouring properties and are inexpensive. Every year, as much as eight million tonnes of synthetic food colours are manufactured for use in the food and beverage industry [1].

Both synthetic dyes and naturally occurring pigments are used as colorants. While synthetic dyes are created chemically, natural colours come from nature and can be extracted from plants, minerals and invertebrates through more or less complex extraction techniques. Most natural colourants have various limitations, including instability in water, reactivity with other food ingredients and odours, and instability when exposed to light and heat.

The vivid colours of candies, sports drinks, and baked items are a result of synthetic food dyes. Even certain brands of pickles, smoked salmon, salad dressing, and pharmaceuticals include synthetic colouring agents. Children are more attracted to plates of food full of colour and are the biggest consumers of synthetic food colour. Consumption of food colours by children has increased by 500 % over the past 50 years [2].

While the use of colouring agents in food has been practiced for ages, the first synthetic colouring agent was made in 1856 using coal tar. Petroleum is now used to make food colouring agents. Numerous artificial food colours have been created over time, the majority of which have since been discovered to be hazardous and banned in many countries. Hence only a limited number of synthetic colours are utilized in the production of food. Analytical methods applied for the detection and quantification of one of the commonest colouring agents, Tartrazine will be discussed in this review.

Azo Dyes

Tartrazine belongs to a group of azo dyes. Azo dyes make up more than 60% of all dyes and they are the dyes used most often. Azo dyes make up over 70% of all industrially used dyes [3].

Azo dyes are chemical compounds that have the functional group diazinyl R-N=N-R', where R and R' can either be alkyl or aryl. Naphthalenes, aromatic heterocycles, benzene rings, or enolizable aliphatic groups can all be linked to the azo group. Due to their range of shade intensities, these are essential for giving the dye its colour. The presence of a highly mutagenic nitro group makes the azo dyes toxic [4].

The chemical structure of azo dye is made up of three groups: auxochrome group, chromophoric group and solubilizing group. A molecule's chromophore is the component that gives the molecule its colour. Auxochromes are atoms that can be bonded to chromophores to intensify their colour.

Tartrazine

Tartrazine (E102), is a water-soluble dye used in foods and beverages to give lemon-yellow colour. It is trisodium salt of tartrazine acid. The majority of food products contain tartrazine as a food colour. The food products with tartrazine may include carbonated drinks, beverages, soups, seasonings, flavourings for fermented milk products, sweets, edible ices, flavourings for processed cheese, flavourings for edible cheese rinds, appetisers, fish roe, fish paste, and crustacean paste, as well as cotton candies, jams, pastry, chips, juices and confectionery like breath freshening and chewing gum.

The Joint FAO/WHO Expert Committee on Food Additives [JECFA] and EU Scientific Committee for Food (SCF) have established 7.5 mg/kg body weight as the Acceptable Daily Intake (ADI) for tartrazine. The highest allowed concentration of all food colours that may be added to various foods in India is 100 ppm, either alone or in combination.

Although Tartrazine adds to the appeal and presentation of foods, it is not without toxic effects, especially if consumed in high quantities. Kamel et al, (2011) reported hyperactivity, anxiety, depression and antisocial behaviour as a result of exposure to tartrazine [5]. Bhattacharjee conducted a study that concluded that reduction in mitotic activity at higher concentration and induction of a number of chromosomal aberrations

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strongly establish the cytotoxic potential of Tartrazine [6]. Oxidative stress, neurobehavioral and haematological changes in rats were reported by Albasher et al. (2020) [1]

In a study on DNA repair tartrazine was found to have significant genotoxic (damaged DNA) effects at all concentrations investigated, but there were no cytotoxic (damaging cells) effects. In the majority of instances, the damage was repairable, but some damage did last in tartrazine-exposed specimens longer than in control specimens, even 24 hours after exposure, according to the in-vitro study. It was determined that persistent tartrazine exposure could cause carcinogenesis [7]. Among other adverse effects of tartrazine reported in humans are Urticaria [8] and hyperactivity in children [9].

This has resulted in the need for methods to detect and measure levels of tartrazine added to food products to ensure the safety of consumers. Tartrazine is a synthetic lemon-yellow dye soluble in water. The ADI of Tartrazine as a food additive is 7.5mg/kg/body weight. However, it is possible that more than a permissible amount of Tartrazine is used to make food items more attractive. This may pose a threat to consumer's health. Therefore, there is a need for a sensitive and robust analytical method to detect and quantitate tartrazine. In the present review analytical methods such as TLC, HPTLC, HPLC, Spectroscopy and Mass Spectroscopy are discussed.

For the present review various scientific research papers and articles published within 2008 to 2022 were referred. The keywords used were 'food colours", "tartrazine", "analytical techniques", "estimation of tartrazine". These scientific papers were sourced from Google Scholar, PubMed Central (PMC), Elsevier and MDPI (Multidisciplinary Digital Publishing Institute). These research papers featured varied subjects like commercial soft drinks, sweets, animal-derived food and feed, herbal medicines, breakfast cereals, soft candies, hard candies, medicinal lollipops etc.

1. Thin Layer Chromatography (TLC)

TLC is an age-old technique used for the identification of tartrazine fa rom mixture since the 1960s. TLC is the simplest chromatographic method not utilizing sophisticated and expensive equipment. The only challenge is selecting the proper stationary phase and mobile phase for the application of dyes.

The TLC method was used to determine synthetic food colour in soft drinks of orange and grape flavours made in Ceará, Brazil by de Andrade et al, (2014). Using C18 SPE (Solid Phase Extraction) cartridges, tartrazine (E102) from soft drinks was extracted and detected by TLC. The concentrations of the food colour were then determined by ion-pair HPLC [10].

TLC method was also used by Rezaei et al, (2015) to determine artificial colours present in food samples taken from several restaurants and confectioneries in the Iranian city of Arak. The Iranian national standard prohibits the use of artificial colours. However, 80% of the food samples that were analysed had artificial colour and 57.1% had tartrazine [11].

The food colours used in Iranian juices and beverages were examined by Gholami et al, (2021). The samples were examined and classified after dye extraction and purification was done using TLC and the retention factor (Rf) was measured. The study's findings revealed that 40% of the samples contained synthetic colours of which 8% had non-permitted synthetic colours including tartrazine [12].

Rajapaksha et al., (2016) carried out a study by taking 120 food samples from Colombo district were analyzed using Thin Layer Chromatography and UV-Spectrophotometry. 85% of samples had permitted colors, with Tartrazine (55.83%) being the most common. Other synthetic colors found were Carmosine (30.83%), Sunset Yellow (25%), Brilliant Blue FCF (20.83%), Ponceau 4R (14.17%), Erythrosine (3.33%), and Allura Red (3.33%). Indigo carmine and Fast Green FCF were absent, but 5.8% of samples contained non-permitted Alizarin [13].

Thus, TLC is the most frequently used method for qualitative analysis and to determine the number of components in a mixture. However quantitative analysis of components in a mixture cannot be done by TLC. For quantification, High Performance Thin Layer Chromatography (HPTLC) an automated form of TLC is used. Other methods used for quantification are Spectroscopy, HPLC and Mass spectroscopy.

2. High Performance Thin Layer Chromatography (HPTLC)

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HPTLC is a robust, quick, easy, and effective technique for analysing compounds quantitatively. Based on TLC, HPTLC is an analytical technique that has been improved to enable quantitative analysis of the compounds and to enhance the resolution of the compounds that need to be separated. HPTLC provides better separation of compounds and a lower limit of detection (LOD).

A high-performance thin-layer chromatography method was used by Soponar et al, (2008) using 3-aminopropyl silica gel, isopropanol, diethyl ether, and ammonia as the mobile phase, combined with image processing, accurately quantified food dyes (tartrazine, azorubine, Sunset Yellow). It demonstrated strong linearity (correlation coefficient: 0.9952–0.9980), low detection limits (5.21–9.34 ng/spot), and minimal equipment costs, making it suitable for routine analysis. Recovery studies showed values between 96.39% and 102.76% [14].

Nambiar et al, (2018) used HPTLC with a single mobile phase to separate and quantify eight food colours including tartrazine and four intense sweeteners. Acetonitrile, water, ethyl acetate, and 10% aqueous ammonia (9:1:1:1, v/v/v/v) were used as the solvent system. The findings demonstrated that all samples contained food colours in the permitted amounts. The method was validated using analytical parameters like linearity, sensitivity, selectivity, precision, accuracy, robustness and stability [15,16,17].

3. High-Performance Liquid Chromatography (HPLC)

Although methods like TLC and Spectroscopy are frequently used to identify water-soluble synthetic dyes, they are either time-consuming or unsuitable for complicated colourant mixes. HPLC is a modified form of column chromatography. Column chromatography consists of a column packed with adsorbent material and a mobile phase is allowed to run down the column by gravity. In HPLC the column is connected to the high-pressure pump and is packed with very fine and uniform pore-size adsorbent material like silica which helps in better separation of mixtures and reduces analysis time. To increase the effectiveness and reliability of the analysis, techniques like reversed-phase liquid chromatography (RPLC) [non-polar stationary phase] as a result non-polar molecules will be adsorbed on silica and polar molecules will elute first], ion-pair RPLC [ions in the samples are paired and separated], and anion-exchange chromatography [ions in the samples are separated as anions and cations separately] for synthetic water-soluble dyes were used Coelho et al, (2009) [18].

Yang et al, (2011) used HPLC connected to a photodiode array detector to identify five food-grade aluminium lake dyes including Tartrazine Al lake dye that can be found in chewing gum [19].

Bonan et al, (2012) simultaneously determined 17 synthetic colours including tartrazine in food and beverages using HPLC and a diode-array detector. The specificity and accuracy (truthfulness and precision) were evaluated. It enabled the processing of up to 20 samples per day and was helpful for regular analysis. Additionally, it enabled the simultaneous determination of red, yellow, and blue food dyes in liquids as well as red and yellow food dyes in solid matrices in a single extraction and chromatographic run. In light of the variety of the matrices examined and the substantial number of analytes, the technique demonstrated good linearity, repeatability, and reproducibility criteria [20].

Elias et al, (2016) used RP-HPLC with a diode array detector to determine 5 synthetic colourants including tartrazine in wine. These food colours were examined using a C18 analytical column within 22 minutes. For the regular examination of food dyes, this approach produced consistent, reproducible results with acceptable detection limits and little processing time [21].

Floriano et al., (2018) used HPLC for identifying 6 synthetic dyes including tartrazine in sports beverages. Relative standard deviation (RSD) was less than 15%, while recovery results of tartrazine ranged between 85 to 90% [22].

Rana et al., (2020) used the HPLC method to detect tartrazine and brilliant blue in commercial formulations. Their detection was done at λmax of 427nm and 629nm respectively. Tartrazine and Brilliant Blue had retention times of 2.847 and 3.512 minutes, respectively. With R^2 of 0.9966 and 0.9957 respectively and a percentage relative standard deviation (% RSD) of under 2%, the approach demonstrated good reproducibility and recovery [23].

Ko et al, (2021) developed the HPLC technique to identify 10 synthetic dyes including tartrazine in Hawthorn fruit, Cornus fruit, and Schisandra fruit. 10 dyes were separated using acetonitrile and 50mM ammonium acetate in distilled water and then separated by gradient elution using a Photodiode Array Detector (PDA) at 428 nm or 500 nm. To confirm the presence of synthetic dyes in the sample solution that tested positive, a new LC-MS/MS method was developed in this study. As a result, HPLC-PDA analysis is regarded as a trustworthy analysis technique that can identify synthetic dyes in herbal medicines [24].

Chen et al. (2019) determined tartrazine in animal-derived foods and feeds using HPLC. Tartrazine was extracted with water, defatted with n-hexane, and cleaned up with weak anion SPE exchange cartridges. On a C18 column using gradient elution, chromatographic separations were carried out. The analyte's average recoveries ranged from 80.4 to 92.5 per cent. The daily and weekly fluctuations were less than 15.0 per cent. This HPLC approach was effective for routinely determining the presence of pigment residues in foods and feeds derived from animals [25].

Rahnama et al., (2022) used HPLC equipped with a UV detector to determine Sunset yellow, quinoline yellow, tartrazine, and other colours in ice creams, freeze popsicles, jellies, and candies. The study also assessed how much of the food colours youngsters consumed. The results showed that out of 107 samples, (7.33%) of the food products had solely tartrazine [26].

Thus, HPLC offers greater potential for qualitative and quantitative analysis of synthetic food colours. Food colours are readily absorbed at UV and visible regions of the electromagnetic spectrum; hence the most common detection method is HPLC using a UV-Visile or diode-array detector (DAD). The best overall sensitivity and resolution are offered by the currently most prevalent technique, RP-HPLC with ion pairing [27,28,29,30,31,32].

4. Spectroscopy

Food colourants are substances that absorb electromagnetic radiation strongly in the visible region of the spectrum. Spectrophotometers are used widely in the determination of food colours. Photoacoustic spectroscopy was used by Coelho et al, (2009) to identify and quantify common food additives such as Brilliant Blue, Synthetic Yellow, and Tartrazine. Photoacoustic spectroscopy utilizes the transformation of radiation (IR, UV) by gases, liquids or solids into acoustic energy. Higher sensitivity and satisfactory precision were achieved using photoacoustic spectroscopy. Other advantages of this method include requiring fewer samples, reusing the same samples and having fewer steps of analysis.

Another spectroscopic technique used in tartrazine estimation is UV-visible spectroscopy. It determines the concentration of analyte in solution by passing a beam of wavelength ranging between 180-1100nm. UV-vis detectors are incorporated into HPLC and ultra-high performance liquid chromatography (UPLC) for measuring the concentration of analytes in complex liquid mixtures.

UV-Visible Spectroscopy was applied by Bachalla, (2016) to extract and identify synthetic food colours added to jams and sweets. Standards and samples for UV-visible spectrophotometry were evaluated for the absorption of synthetic food colours. The result showed that the spectrophotometric method can be used for the qualitative detection of tartrazine [33].

Another study by Dilrukshi et al., (2019) identified the synthetic food colourants used in the drinks and sweets sold in the Jaffna district of Sri Lanka. Thin-layer chromatography was used to identify extracted colour and UV-visible spectrophotometry was used to recognize unidentified spots on the TLC plate. Tartrazine was detected in 41% of sweets and beverages. The study concluded that confectioneries and beverages have a significant tendency to employ synthetic food colours, especially tartrazine [34].

In yet another study, Rajpaksha et al, (2015) determined non-permitted colours including tartrazine in food and beverages using the TLC method and UV-Spectrophotometric method. The TLC technique was primarily used to detect the artificial colours added to food, and the UV-spectrophotometric approach was subsequently used to validate the presence of colours that were not permitted [35].

The level of adulteration with synthetic colourants (Tartrazine, Fast Green FCF and Sunset Yellow) was assessed by Siddartha et al., (2022). The UV-VIS data revealed that the amount of tartrazine used was excessive, exceeding the Maximum Permissible Limit. UV-visible spectrophotometers are used widely in the determination of food colours because of their low

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instrumentation cost compared to mass spectroscopy [36].

In the study carried out by Siddarth et al., by collecting of 40 milk-based sweets, Tartrazine was found to exceed the maximum permissible level, averaging 517mg/kg according to UV-VIS spectrophotometry. UV-VIS showed better accuracy in estimating colour concentration, particularly for turbid extracts, compared to a normal spectrophotometer [37,38,39].

5. **Liquid Chromatography/ Mass spectroscopy (LC/MS)**

It has always been desired to combine mass spectroscopy with chromatographic methods since MS is more sensitive and highly specific than other chromatographic detectors. It can handle complex mixtures of food colours and has a high level of specificity.

Tsai et al., (2014) created LC-MS/MS method to detect 20 synthetic food colourants in food products, including tartrazine. In this study, a single-step extraction methodology for an LC-MS/MS approach was devised for determining the presence of dyes in food. The newly created SPE cartridges when using LC-MS/MS [40].

Shamari et al., (2020) used LC-MS for determining tartrazine in carbonated beverages. In a two-step method: the first includes method development to identify dyes, and the second involves solid-phase extraction before application. The LC-MS approach can be used to quantitatively estimate tartrazine and is quick and accurate [41].

CONCLUSION

Tartrazine is a lemon-yellow synthetic azo dye used to give foods and beverages a bright lemon-yellow colour. However, there are several recognized adverse effects of tartrazine in humans when used in excess quantities. Due to the growing use of tartrazine in the production of foods, there is a need to develop a sensitive, precise, accurate and selective analytical method to detect and quantify the Tartrazine content to ensure that the colouring agent is used within permissible limits. We have reviewed different analytical techniques employed for the determination of tartrazine. Each of the described methods except TLC can be used to quantitate tartrazine. Out of all the techniques discussed TLC is the most frequently used technique in qualitative determination of tartrazine. However, TLC alone cannot be used for the estimation of tartrazine. Sensitive and specific methods such as UV-visible spectroscopy, HPLC and HPTLC are frequently used for quantitation of tartrazine in food items. Sensitive and specific methods of detection of Tartrazine are critical for control of excessive use of the food colour Tartrazine.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

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