



Analytical Approaches to Food Dye Analysis and Detection of Dye Adulteration in the Food, Supplement, Animal Nutrition, Cosmetics, and OTC Pharmaceutical Industries



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SUMMARY:

With changes in the supply chain and an increased demand for natural products, it is becoming more and more important to test products and ingredients for synthetic food dyes. Synthetic dyes can be used in place of natural color options, or they can be used to make a cheap ingredient look like a more expensive one. Products that claim to only have natural colors that are suspected to have synthetic dyes can be detained by the FDA, resulting in extremely expensive recall testing before return to the market. Even when a company thinks they are using natural colors, it is essential to test ingredients for synthetic dyes to avoid complications down the road.

INDUSTRIES WHICH USE FOOD DYES AND/OR HAVE POTENTIAL FOR ADULTERATION:

Herbal products: In 2021, The EU published a report investigating 6 herbal products: pepper, chili, curcuma, cumin, saffron, and oregano. They found a range from 0.0% (pepper)–8.5% (saffron) of samples investigated contained non-authorized dyes¹. Botanicals, especially vibrant spices are a target for food dye contamination and adulteration because their natural hue is often lost during processing. It is especially important to screen for unauthorized dyes in herbal raw materials and post-processed products to ensure their purity and safety.

Processed foods and adult nutritionals: Many finished products, like gummies, capsules, candies, beverages, etc., may have a particular color that consumers associate with a flavor or scent. Food color is an important component of a product, so producers often use natural or synthetic dyes to maintain visual appeal². While using FD&C colors (detailed below) is legal and regulated, issues arise when a product is labeled as “natural” but contains synthetic dyes. There is also evidence of lot-variability of dye quantity in food which can result in color, health, and quality inconsistencies³. To avoid future regulatory and quality control issues, it is best to screen for known and unknown food dyes prior to putting a product on the market.

Animal nutrition: Animals can rarely perceive color the way a human can, so adding food dyes to pet food and animal feed may seem unnecessary. However, many companies add coloring agents to pet food to improve attractiveness to the humans buying the products. It is critical to list all dyes, synthetic or natural, in animal nutritional products⁴. Consumers are becoming more aware of potential risks associated with food dyes and deserve the truth about the products provided to their pets.

Other industries:

- o Cosmetics
- o Dietary supplements
- o OTC pharmaceuticals

THREE MAIN CATEGORIES OF FOOD DYES:

Food, Drug, and Cosmetic (FD&C) dyes: FD&C dyes are synthetic dyes that are clearly defined and regulated by the FDA. Companies who synthesize these dyes are required to submit each batch to an FDA certified lab to inspect the chemical composition, purity, and other regulatory aspects prior to sale. In most cases, food, drug, and cosmetic producers buy dyes from authorized companies to add to their products. This means that product developers rarely test the dye itself, and instead screen for common FD&C dyes to confirm they are not present

in raw materials or in any products that claim to be “all natural.” Most analytical testing for food dyes screen for FD&C dyes because they are the most common adulterant of natural colors and are well characterized and easy to detect. Table 1 outlines the 9 FD&C dyes approved for use in food products and an additional 14 approved for use in drugs and cosmetics.

Table 1: List of FDA approved synthetic dyes for use in Food, Drugs, and Cosmetics. Note that dyes not approved for use in food are named D&C only⁵.

Table 1: List of FDA approved synthetic dyes			
Name	Food	Drugs	Cosmetics
FD&C Blue No. 1	•	•	•
FD&C Blue No. 2	•	•	
FD&C Green No. 3	•	•	•
Orange B	•		
Citrus Red No. 2	•		
FD&C Red No. 3	•		•
FD&C Red No. 40	•	•	•
FD&C Yellow No. 5	•	•	•
FD&C Yellow No. 6	•	•	•
D&C Black #2			•
D&C Black #3			•
D&C Green #5		•	•
D&C Orange #5		•	•
D&C Red #6		•	•
D&C Red #7		•	•
D&C Red #21		•	•
D&C Red #22		•	•
D&C Red #27		•	•
D&C Red #28		•	•
D&C Red #30		•	•
D&C Red #33		•	•
D&C Red #36		•	•
D&C Yellow #10		•	•

Natural food colors: Technically, there is no legal definition for the term “natural food dye.” The FDA broadly classifies colorants as FD&C (meaning they are synthetic and batch testing is required) or not. To a consumer and most producers, natural colors are any source of coloring from a plant or environmental source, like fruit and vegetable juice, extracts, minerals, or animal products⁶. A common example is anthocyanins, which are plant compounds ranging in color from blue to red. While natural colors are considered the safe alternative to synthetic dyes and are commonly sought by consumers, there are many challenges associated with their use. These issues include altering the odor or flavor of a product, instability, and high extraction costs. It is difficult to screen for all possible natural color sources, but it is generally expected that ingredient lists contain natural food colors added

to specifically enhance a product's appearance. Manufacturers should be aware of the nuanced labeling rules around how coloring is declared on front of pack as well as within ingredient declarations and seek consultation if unsure. Eurofins Food Assurance offers such services.

Many tests for natural food dyes overlap with procedures used for vitamin and dietary supplement testing. Although variation and instability makes natural color analysis more difficult, well designed analysis can characterize the natural colors present and accurately measure their stability through shelf life and processing. Accurate HPLC tests are available for many pigments including beta-carotene, lutein, zeaxanthin, bixin, astaxanthin and numerous anthocyanins.

Unauthorized dyes: Dyes that are not approved for use by the FDA can be harmful, but they are very common in spices and herbal products. Typically, unauthorized dyes are synthetic and are much harder to test than FD&C dyes. A main problem with screening for unauthorized dyes is their chemical composition is often unknown. Sudan dyes are the most common class of unauthorized dyes, which are added to spices like chili powder to increase the bright red hue customers expect.

A category of food dyes that contains both FD&C regulated colors and unauthorized dyes are Azo dyes. Azo dyes are named after the nitrogen containing functional group housed within their structures (-N=N-). Azo dyes in their natural form are non-toxic, but are reduced in the intestines to form aromatic amines correlated with neurotoxicity, genotoxicity, and carcinogenicity. While most azo dyes are banned internationally, at least 4 have some approved uses in the United States: E102 (Tartrazine, FD&C Yellow 5), E110 (Sunset, FD&C Yellow 6), E121 (Citrus Red 2, only approved for coloring orange peels), and Orange B (only approved for coloring hot dog and sausage casings). Screening for azo dyes ensures products meet all regulatory and legal requirements and that all colorants in a product can be properly disclosed to consumers.

POTENTIALS FOR ADULTERATION:

Any product manufactured from out-of-house raw materials has potential for food dye contamination. If you source raw materials from any external company, there is potential that it has been adulterated in order to improve the visual appearance or decrease production prices. This is especially true for herbs and spices, whose vibrant colors are desired and used as an identification standard.

Food dye adulteration is dangerous. Harmful, unregulated synthetic compounds can have adverse effects on consumers, and with increasing chemical compounds used for dyes, researches cannot keep up with their safety.

As the health community grows, more consumers are reading labels to avoid food dyes and synthetic additives. When a label claims there are no additives, it is important that claim is valid and can be backed by the proper testing. Importantly, this testing should be done prior to putting the product on the market—if a product is investigated by the FDA for fraudulent claims surrounding food dyes, the resulting regulatory and laboratory costs to prove the claims are steep. Testing beforehand reduces the risk of recall and the cost of subsequent testing.

BENEFITS OF FOOD DYE TESTING:

- o Prevent potential adulteration with unknown or unexpected food dyes
- o Confirm ingredient claims
- o Improve consumer trust and return
- o Prevent FDA detainment
- o Reduce costs in the case of FDA investigation

TYPES OF PRODUCTS TO TEST:

Food dyes are used in virtually every food, supplement, cosmetic, and animal food matrix, so scientists have developed methods to test many kinds of products including:

- o Raw materials
- o Finished products, including solids, powders, and capsules
- o Gummies
- o Liquids and beverages
- o Purified extracts and tinctures

It is important to consult with your product testing team to discuss the best fit-for-practice approach to testing your specific product. Different formulations and matrices require different extraction methods. Solvent choice, extraction technique, and processing procedures can all impact the analytical outcomes of testing.

Methods for food dye and natural colorant extractions include:

- o Filtration and centrifugation of liquids
- o Solid phase extraction
- o Liquid-Liquid extraction
- o Microwave assisted extraction

TYPES OF ANALYTICAL TESTS:

Spectrophotometry:

Spectrophotometry uses specific wavelengths of light to measure the quantity of a class of compounds (which have specific absorption and transmission wavelength ranges). The amount of light transmitted through a sample is used to quantify the amount of a compound that was available to absorb light. This is very useful for food dye analysis because highly pigmented compounds are more light sensitive. Spectrophotometric approaches are straightforward and user friendly and their low costs and quick results make them popular for colorant testing. However, there are limitations in specificity, meaning the specific compounds responsible for absorbance are unknown and synthetic and natural dyes can rarely be distinguished.

Thin Layer Chromatography (TLC):

TLC is based on the principles of chromatography: compounds of different weights and polarity move through space at different speeds. In TLC, samples are prepared and plated on a thin silica-gel plate and various solvents are used to separate compounds based on their weight, size, and affinity to the solvent. Each compound has a specific pattern, so food dyes can be compared to purified standards to access their presence. TLC is an increasingly common, high-throughput technique that is quick, efficient, and reproducible.

High Performance Liquid Chromatography with UV detection (HPLC-UV):

HPLC uses the same ideas of TLC—separation of compounds based on their weight and affinity to a solvent. With HPLC for dye testing, samples are prepared in a suitable solvent and run through a column that can pull apart very polar compounds with similar structures—the case for most synthetic and natural dyes. After separation, sample fragments are sent to a detector which uses UV light to detect the presence of compounds that absorb specific wavelengths. HPLC-UV can detect low levels of the synthetic compounds, and is a straightforward approach to detecting adulteration. In addition to detecting the presences of expected dyes, HPLC-UV can measure their concentration. This can be used as a tool in standardizing the concentration of natural colorants like anthocyanins where there may be natural variations in the color intensity of a fruit or vegetable concentrate.

Liquid Chromatography – Mass Spectrometry (LC-MS or LC-MS/MS):

LC-MS is one of the most common techniques used in botanical testing. Following liquid chromatography separation, compounds are ionized and the mass of ionized form is measured, and further fragmented into additional ions. This provides structural information to identify individual compounds, improving the resolving power and identification potential. The ability to identify individual compounds allows researchers not only to determine if there is food dye adulteration, but also to notify companies of the exact dye in their products. Testing for natural food colors largely overlaps with vitamin and dietary supplement testing because the same compounds that provide color also provide nutritional benefits and are key marker compounds.

WHERE TO TEST:

Food dye testing can be performed in house or by an independent, third party lab. While internal testing may save time and money, there are limitations, such as underqualified analysts, non-specific methods, and problems with troubleshooting. Using an independent lab, like Eurofins, provides expert advice, personalized method development, data analysis, and detailed knowledge of federal and state regulations.

Internal

Internal lab testing provides options for companies to factor in routine investigations and approaches into their production time, without having to rely on another company's testing speed. This can improve the consistency of testing and product development as well as shorten the time for raw material acceptance and/or product release. However, it is important that internal testing is highly standardized and reliable, with trained scientists performing tests according to standardized procedures and a robust quality management system (QMS). Internal testing has the benefit of deep knowledge of the product, but that also introduces heavy bias in the interpretation of results and outcomes.

Independent

Independent labs are staffed with well-trained, experienced scientists with expertise in analytical chemistry, method development, and product formulations. This means that no matter the product and concern, there will be someone who knows how to test it. This is a huge benefit, especially as problems arise in the testing workflow. Experienced and knowledgeable scientists are well-versed in troubleshooting and resolving issues with methods and instruments quickly, ensuring reliable and timely results. Additionally, testing in an external lab means product manufacturers do not have to search for, train, or pay scientists themselves specifically for food dye testing, not to mention the significant cost to maintain instruments and infrastructure, as well as maintain stocks of costly standard, reagents and lab supplies that independent labs can often purchase in bulk and at significant discounts.

On top of a staff of scientists, there are other benefits and considerations to using a third-party lab for testing. These include:

- o Efficient, standardized approaches to data collection and sharing
- o Communication with scientific experts and detailed information about procedures
- o Little restriction in possible instrumentation and testing abilities
- o Fast and predictable turnaround time
- o Independent Quality Management System Accreditations such as ISO-17025

CONCLUSION:

Natural and synthetic dyes are used in almost all industries, ranging from cosmetics to animal feed. It is important to accurately screen for dyes to ensure label compliance, product safety, and ingredient claims. Understanding the potential for dye adulteration is critical to knowing how and why products are affected. Furthermore, testing with a third-party lab can lead to standardized product coloration and reliable ingredient claims. The scientists at Eurofins are excited to work with you to develop the perfect food dye testing approach for your needs!

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