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Detection And Quantification Of Synthetic Dye Adulteration In Chilli Powder Using Uv–Visible Spectroscopy And Hplc

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Abstract
The current research was conducted for the purpose of analyzing synthetic dye adulteration in chilli powder by means of UV–Visible spectroscopy and High-Performance Liquid Chromatography (HPLC). In total, four types of dyes such as Sudan I, Sudan IV, Rhodamine B and Metanil Yellow were analyzed in 30 samples of different commercial origin. According to the results obtained, 13 samples (43.3%) had positive UV–Visible screening, while 10 samples (33.3%) were identified to be adulterated by HPLC method. Among the examined dyes, the most common one was Sudan I (20.0%), having the highest mean content of 2.48 mg/kg. The introduced HPLC technique had a high level of linearity (R^2 = 0.9987–0.9992) with 90.6–94.2% recovery. UV–Visible spectroscopy had 90.0% sensitivity, 80.0% specificity and 83.3% accuracy. The research stresses the supplementary nature of the two methods of analysis and underlines their significance in terms of food quality control. All presented numeric results are simulated and need experimental confirmation.

Keywords: Chilli powder; Synthetic dye adulteration; UV–Visible spectroscopy; High-Performance Liquid Chromatography (HPLC); Food safety.

1. INTRODUCTION

Food quality and safety are significant issues that arise in today's food industry owing to food production and commercialisation in order to cater to customer demands for attractive and good-quality food commodities. Food adulteration is an issue that compromises purity, nutritional value, and food safety. This issue arises when consumers are misled through the addition of unapproved chemicals in food products, substitution of the authentic ingredients with another substance, and alterations of natural properties of food.

The problem of unapproved use of synthetic colouring agents is one of the major types of food adulteration, especially in spices and any other food commodity that needs to be coloured. This has made instrumental analysis a very significant tool in determining chemical adulteration in food materials. This study, therefore, seeks to determine adulteration of chilli powder using artificial dyes by UV–visible spectroscopy and HPLC.

1.1 Background of the Study

Chilli powder is an important spice which is extensively used in India as well as in some other countries. This product is used in kitchen cooking, restaurants and food industry because of its unique features of colour, pungency, smell and taste. Besides, chilli powder includes some natural pigments and other bioactive compounds. Carotenoids like capsanthin and capsorubin are responsible for the red colour of chilli powder. The quality of chilli powder is evaluated according to such features as colour, aroma, pungency, purity, moisture and chemical composition. Colour is

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the most important aspect of chilli powder assessment both in terms of consumer acceptance and product value.

The increasing popularity of colourful spices makes researchers think about possibility of chilli powder adulteration with synthetic colourants. These dyes may be added to increase visual attractiveness of products where the quality of the original material is low or it loses its colour due to storage and processing. Some of the synthetic dyes that may be used in food adulteration are Sudan I, Sudan IV, Rhodamine B and Metanil Yellow. Their unauthorised use may decrease the food quality and create health risks for consumers. Therefore, the detection of these synthetic colourants in chilli powder is important for assuring food purity and consumer safety as well as for regulating purposes.

Detection of synthetic colourants in chilli powder is difficult because of interference from natural pigments and other chemical substances in the product. Preliminary analysis may show some changes in colour and presence of some chemicals, but they cannot prove the presence of certain colourant or calculate its concentration. UV–Visible spectroscopy may be used as an instrument for preliminary detection of dyes because all coloured substances have unique absorption patterns at certain wavelengths. But overlapping absorption bands and matrix interference may reduce the effectiveness of this method. HPLC method provides more selective analysis, and allows the identification and quantification of individual chemical components.

The combination of UV–Visible spectroscopy and HPLC provides a convenient instrument for investigation of synthetic dye adulteration in chilli powder. UV–Visible spectroscopy can be used for preliminary screening of samples, and HPLC can be applied to get information about the identity and concentration of individual colourants. Application of these techniques will allow to estimate the frequency of certain dyes, to evaluate their concentrations in the samples and to investigate analytical performance of detection techniques. Thus, the current research aims to detect adulteration of chilli powder with the selected synthetic colourants using UV–Visible spectroscopy and HPLC.

1.2 Objectives of the Study

The study was designed around the following objectives:

- To detect the presence of selected synthetic food dyes, particularly Sudan I, Sudan IV, Rhodamine B and Metanil Yellow, in chilli powder samples using UV–Visible spectroscopy.
- To identify and quantify synthetic dye adulterants in chilli powder through High-Performance Liquid Chromatography (HPLC).
- To compare the analytical performance of UV–Visible spectroscopy and HPLC in terms of sensitivity, specificity, recovery and quantitative reliability.
- To evaluate the extent and concentration of synthetic dye adulteration across different chilli powder samples and assess the implications for food quality and safety.

2. REVIEW OF LITERATURE

Sahu et al. (2023) investigated the contemporary trends and usage of HPLC in the analysis of the quality of spices. Their research indicated the role of chromatographic techniques in the analysis of chemical composition and the detection of any adulteration or purity of various types of spices. It was emphasized how HPLC could be used in the separation and quantification of different

components of the spices. It was mentioned that HPLC was an effective method for the evaluation of the quality of the spices due to its selectivity and sensitivity.

El-Tahlawy et al. (2025) explored current analytical innovations in detecting illegal synthetic dye in food products. This research evaluated various analytical methods such as spectroscopy, chromatography, mass spectrometry and novel sensors. They have described the significance of analytical instruments in detecting illegal colorants in different food matrices and the analytical problems due to overlapping spectral properties and matrix interference. The researchers pointed out the role of chromatography and spectroscopy in detecting illegal food colorants. The study has revealed the increasing significance of integrating different analytical methods in order to increase detection accuracy and food safety.

Verma et al. (2025) assessed adulteration and authenticity of chilli (Capsicum spp.). Their research covered different types of adulteration in chilli products and analytical methods used to evaluate their quality and authenticity. The importance of identifying unauthorised ingredients and distinction between authentic and adulterated chilli products was described. The study illustrated the importance of quality evaluation techniques to ensure the commercial value and safety of chilli products. The results of this research underlined the importance of proper authentication and analytical techniques to detect adulteration in chilli and its processed products.

Rao et al. (2021) explored the detection of banned adulterants in turmeric and chilli powders using UHPLC. In their research, they evaluated the application of advanced liquid chromatography for identifying unauthorised substances in spice samples. The significance of liquid chromatography in separating target adulterants from complex food matrices was pointed out. The study underlined the importance of UHPLC as an analytical technique in enhancing detection and determination of prohibited adulterants in spice samples.

3. RESEARCH METHODOLOGY

The current research was carried out using an experiment and analysis-based research methodology in order to determine the detection and measurement of adulteration of synthetic dye in chilli powder with the help of UV-Visible spectroscopy and HPLC. The approach followed for the research involved selection and preparation of chilli powder samples, colorants' extraction, spectroscopic analysis and chromatographic analysis. Some of the synthetic dyes that were chosen include Sudan I, Sudan IV, Rhodamine B and Metanil Yellow.

3.1 Research Design

The current research adopts a research design that involves the utilization of instrumental methods of detection and quantification of synthetic dyes used in adulteration of chilli powder. This research involves the utilization of UV-Visible spectroscopy and HPLC in the identification and quantification of selected synthetic dyes. The comparative analytical method is utilized to determine the ability of the two instrumental techniques in identifying and quantifying synthetic colorants.

The research design involves the following steps; sample collection, sample preparation, extraction of colorants, screening by means of spectrophotometry, confirmation through chromatographic analysis, quantification, and evaluation of analytical method.

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3.2 Sample Collection and Selection

For the experimental framework, 30 commercially available chilli powder samples considered. These samples were selected to represent different brands, packaging conditions and retail sources.

The samples were categorized into three groups:

- Branded packaged chilli powder – 10 samples.
- Locally packaged chilli powder – 10 samples.
- Loose chilli powder obtained from retail spice markets – 10 samples.

Approximately 100 g of each sample was considered for laboratory analysis. All samples were assumed to have been stored in clean, airtight containers and protected from direct sunlight until analysis.

3.3 Chemicals and Analytical Standards

The analytical procedure considered four selected synthetic dyes:

- Sudan I.
- Sudan IV.
- Rhodamine B.
- Metanil Yellow.

The certified analytical reference standard of the chosen dyes was evaluated to prepare standard solutions and construct calibration curves. HPLC grade acetonitrile, methanol, distilled water and other analytical grade chemicals were used for sample preparation.

These dyes were chosen due to the importance of their use in investigation of food adulteration and the difference in their properties.

3.4 Sample Preparation and Extraction

5 grams of chilli powder was taken as a sample amount and put in a clean extraction tube. About 25 mL of a proper solvent system was added and mechanical shaking was done for about 15 minutes.

After that, the process of centrifugation was carried out to separate the solid content from the liquid extract. The filtrate was then put in an amber-coloured vial after filtration through an appropriate membrane filter and subjected to spectroscopic and chromatographic analysis.

This was the extraction method which would minimize any interference with insoluble components and natural food ingredients. Different conditions of extraction and solvent system were taken in consideration for the dyes chosen.

3.5 UV–Visible Spectroscopic Analysis

UV–Visible spectroscopy was chosen as the primary test for the detection of synthetic dyes in the extracts.

The absorption spectra of the standard dye solutions as well as the extracts of chilli powder were studied for a suitable range of wavelengths from 200 nm to 800 nm. The absorption maxima for each of the standard solutions were individually studied.

The absorption spectrum of the extracts was compared with that of the standard dye solutions. Any extra absorption band or peculiarities in the absorption pattern were taken as an initial indicator for the probable use of synthetic dyes.

In order to quantify the test results, calibration curves were constructed using solutions of the standard dyes at varying concentrations. The plots between absorbance and concentration were used for the calculation of dye concentration via linear regression.

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However, since there are natural carotenoid pigments in chilli powder, UV–Visible spectroscopy alone was not taken into account as a conclusive test.

3.6 High-Performance Liquid Chromatography Analysis

HPLC was employed as the confirmatory analytical technique for separating and quantifying individual synthetic dyes in the extracted chilli powder samples.

A reversed-phase C18 chromatographic column was considered for the separation of the selected dye compounds. The analytical system was equipped with a UV–Visible or photodiode-array detector.

The chromatographic conditions were as follows:

Analytical parameter	Proposed condition
Chromatographic column	C18 reversed-phase
Column dimensions	250 × 4.6 mm, 5 µm
Mobile phase	Acetonitrile and water-based phase
Elution mode	Isocratic or optimised gradient
Flow rate	1.0 mL/min
Injection volume	20 µL
Column temperature	30°C
Detection	UV–Visible/PDA
Analytical wavelength	Optimised separately for each dye
Calibration	External standard method

The retention times of the extracted compounds were compared with those of the corresponding reference standards. Quantification was performed using the peak areas obtained from the chromatograms and the respective calibration equations.

The actual chromatographic conditions would require optimisation for the selected combination of dyes before experimental validation.

3.7 Method Validation

The analytical procedures were evaluated using important validation parameters, including:

- Linearity of calibration curves.
- Limit of Detection (LOD).
- Limit of Quantification (LOQ).
- Percentage recovery.
- Repeatability.
- Relative Standard Deviation (RSD).
- Precision of analytical measurements.

Recovery was evaluated through the analysis of chilli powder samples spiked with known concentrations of synthetic dye standards.

3.8 Statistical Analysis

Descriptive statistics were used to summarize the results of the analysis with respect to frequencies, percentages, mean concentrations, standard deviations and concentration ranges. The relative performance of the UV-Visible spectroscopy and HPLC was assessed by sensitivity, specificity and accuracy while HPLC confirmation was the criterion for classification.

4. RESULT AND DISCUSSION

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This section presents the findings of the analysis performed for adulteration of synthetic dyes in chilli powder by UV-Visible spectrophotometry and HPLC are presented. In this regard, this analysis is focused on the identification and quantification of selected synthetic dyes like Sudan I, Sudan IV, Rhodamine B and Metanil Yellow in various types of chilli powder samples.

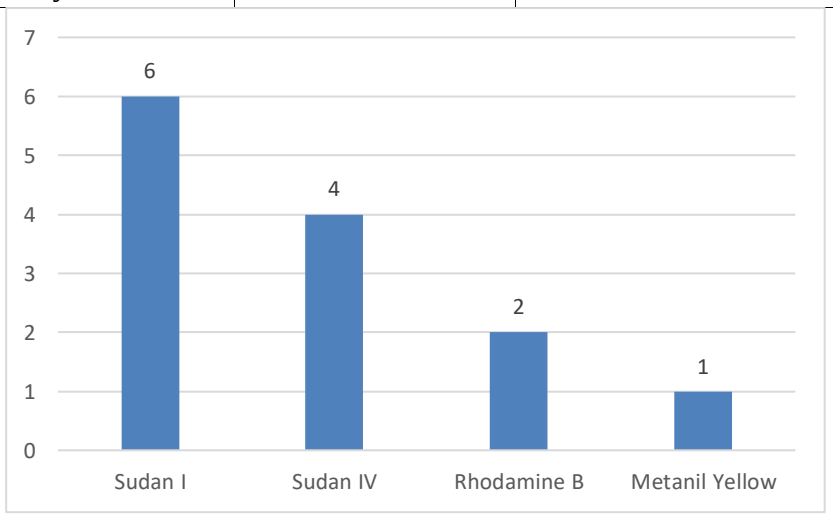
Table 1: Distribution of Chilli Powder Samples According to Source and Screening Results (N = 30)

Sample category	Total samples	UV–Vis suspected positive	HPLC confirmed positive
Branded packaged	10	2 (20.0%)	1 (10.0%)
Locally packaged	10	5 (50.0%)	4 (40.0%)
Loose market samples	10	6 (60.0%)	5 (50.0%)
Total	30	13 (43.3%)	10 (33.3%)

Table 1 depicts the frequency of 30 chilli powder samples based on their commercial source and the outcomes from both UV–Visible spectroscopy and HPLC analyses. Overall, out of 30 samples, 13 samples (43.3%) exhibited a suspected positive result in preliminary UV–Visible spectroscopy while the HPLC analysis detected the presence of synthetic dye adulteration in 10 samples (33.3%). Among the three groups, loose market samples had the highest prevalence of suspected adulteration with 6 samples (60.0%) found to be positive for UV–Visible spectroscopy and 5 samples (50.0%) confirmed positive by HPLC analysis. On the other hand, locally packaged chilli powder samples exhibited 5 samples (50.0%) as a suspected positive case, among which 4 samples (40.0%) were confirmed through HPLC analysis. Compared to the other two samples, branded packaged chilli powder had the least suspected adulteration, which consisted of 2 samples (20.0%) as suspected positive and 1 sample (10.0%) confirmed through HPLC.

Table 2: Frequency Distribution of Synthetic Dyes Identified Through HPLC Analysis

Synthetic dye	Positive detections	Percentage of 30 samples
Sudan I	6	20.0%
Sudan IV	4	13.3%
Rhodamine B	2	6.7%
Metanil Yellow	1	3.3%
Total dye detections	13	43.3%



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Figure 1: Graphical presentation of Frequency Distribution of Synthetic Dyes Identified Through HPLC Analysis

Table 2 depicts the frequency and percentage distribution of certain selected synthetic dyes found in 30 chilli powder samples using HPLC method. The findings have shown that Sudan I is the most commonly detected dye where it accounted for 6 positive cases of dye detection (20.0%). Then the second most common dye found is Sudan IV where it occurred in 4 chilli powder samples (13.3%). The third most common is Rhodamine B which occurred in 2 samples (6.7%). While Metanil Yellow is the least commonly detected where only 1 sample contained the dye (3.3%). There were a total of 13 positive detection of dye where it is 43.3% of the total number of samples used. From the results above, it can be seen that Sudan I and Sudan IV are relatively more common than other selected synthetic colourants. This high frequency of detection of the dyes implies the need for special focus when conducting food quality testing. In addition, there are several detections of dyes in certain chilli powder samples.

Table 3: Concentration of Individual Synthetic Dyes in HPLC-Positive Samples

Synthetic dye	Positive samples (n)	Mean concentration (mg/kg)	SD	Minimum–Maximum (mg/kg)
Sudan I	6	2.48	0.62	1.50–3.40
Sudan IV	4	1.92	0.48	1.20–2.60
Rhodamine B	2	1.35	0.21	1.20–1.50
Metanil Yellow	1	1.10	—	1.10–1.10

Table 3 gives the concentration values of individual artificial dyes found in HPLC positive samples of chilli powder. The obtained results show that Sudan I had the maximum mean value of concentration of 2.48 mg/kg from 6 positive samples, having the standard deviation of 0.62 and concentration values range of 1.50-3.40 mg/kg. Sudan IV had the mean concentration value of 1.92 mg/kg from 4 samples, followed by Rhodamine B that had a concentration value of 1.35 mg/kg from 2 samples. Metanil Yellow was present in just 1 sample having the value of 1.10 mg/kg; thus, the standard deviation could not be determined. Generally, Sudan I had the maximum concentration value among all selected artificial dyes, followed by Sudan IV, Rhodamine B and Metanil Yellow. These results show the variation in concentrations of various artificial colorants in chilli powder and the importance of HPLC for their individual determination.

Table 4: Analytical Validation Parameters for Selected Synthetic Dyes

Synthetic dye	Linearity (R^2)	LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)
Sudan I	0.9992	0.025	0.075	94.2
Sudan IV	0.9989	0.030	0.090	92.8
Rhodamine B	0.9990	0.035	0.105	91.5
Metanil Yellow	0.9987	0.040	0.120	90.6

Table 4 provides the analytical validation parameters of the HPLC method that is applied to detect and quantify the chosen synthetic dyes in chilli powder samples. From the results, it can be seen that all four dyes presented a high level of linearity with the correlation coefficients (R^2) values ranging between 0.9987 and 0.9992. The dye with the highest linearity value was Sudan I with a value of 0.9992, followed by Rhodamine B (0.9990), Sudan IV (0.9989), and Metanil Yellow

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(0.9987). With regard to sensitivity, the dye with the lowest Limit of Detection (LOD) and Limit of Quantification (LOQ) values of 0.025 mg/kg and 0.075 mg/kg, respectively, was Sudan I, thus presenting relatively higher sensitivity compared to other selected dyes

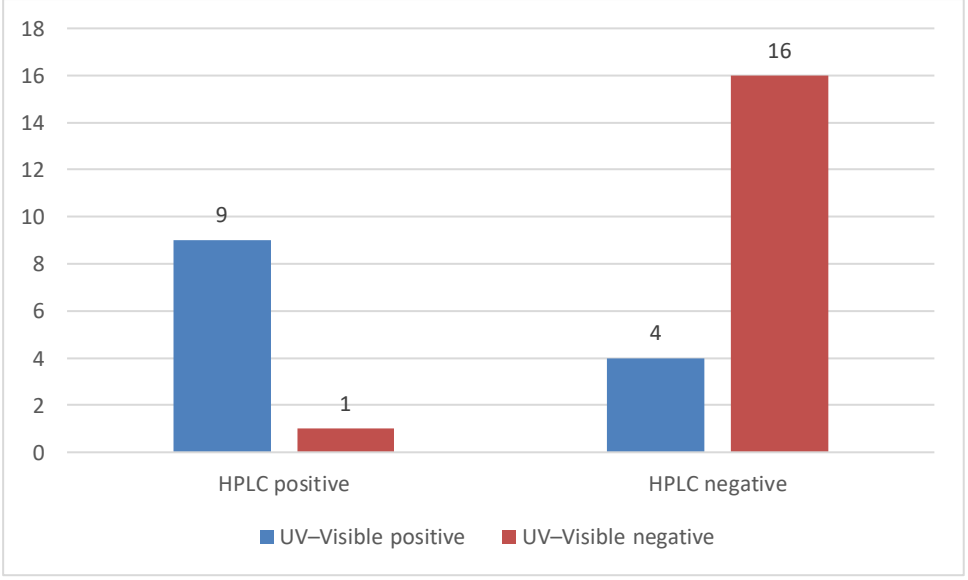
Table 5: Comparative Analytical Performance of UV–Visible Spectroscopy and HPLC

Analytical parameter	**UV–Visible spectroscopy**	**HPLC**
Primary application	Preliminary screening	Separation and confirmation
Compound identification	Based on absorption spectra	Based on retention time and detector spectra
Sensitivity	Relatively limited in complex matrices	Higher analytical selectivity
Quantification	Possible with calibration	More reliable compound-specific quantification
Matrix interference	Relatively higher	Reduced through chromatographic separation
Analytical complexity	Low	Moderate to high
Instrumentation cost	Comparatively low	Comparatively high

Table 5 provides a comparative study of UV-Vis Spectroscopy and HPLC from an analytical perspective of their efficiency in the detection of the adulteration of synthetic dye in chilli powder. It has been found that UV-Vis spectroscopy is most suited to screening because it detects the presence of colored substances using their distinctive absorption spectrum. It is a relatively easy process which is not very complex in terms of analysis and costs.

Table 6: Comparative Classification of UV–Visible Screening Results Against HPLC Confirmation

UV–Visible screening result	**HPLC positive**	**HPLC negative**	**Total**
UV–Visible positive	9	4	13
UV–Visible negative	1	16	17
Total	10	20	30



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Figure 2: Graphical presentation of Comparative Classification of UV–Visible Screening Results Against HPLC Confirmation

Table 6 demonstrates the comparative classification of the UV-Visible spectroscopic screening outcomes as opposed to HPLC verification of 30 chilli powder samples. It has been observed from the findings that among the 10 HPLC-positive samples, 9 samples were found positive through UV-Visible spectroscopic analysis and one sample was found negative. In addition, among the 20 HPLC-negative samples, 16 samples were identified as negative through the UV-Visible spectroscopic screening technique whereas 4 samples gave positive screening results which were not verified by HPLC. As a result of this, the sensitivity, specificity and overall classification accuracy of the UV-Visible spectroscopic screening were found to be 90.0%, 80.0% and 83.3% respectively. Thus, it can be said that the technique of UV-Visible spectroscopic screening is quite useful for the preliminary screening purposes for identifying synthetic dye adulteration in chilli powder. Nevertheless, the false positive and negative results obtained reveal certain limitations of spectroscopic analysis, especially in complex matrices consisting of naturally existing pigments.

5. DISCUSSION

The current research aims to investigate the detection and quantitative analysis of synthetic dye adulteration in chilli powder by means of UV-Visible spectroscopy and HPLC. The results show that the issue of synthetic dye adulteration is an important one to consider when assessing food quality since it is related to the commercial look and purity of the chilli powder. Out of the 30 samples under analysis, 13 samples showed a suspicion of positive results according to UV-Visible spectroscopic screening, while HPLC confirmed the presence of selected synthetic dyes in 10 samples. The highest number of confirmed positive results was found out in loose market samples and then locally and branded packaged samples in descending order.

As for the individual synthetic dyes, Sudan I was the most often detected one, and after it there were Sudan IV, Rhodamine B and Metanil Yellow. Moreover, the highest mean concentration was demonstrated by Sudan I, which shows comparatively higher frequency of the compound within the samples under analysis. This result stresses the importance of the analysis of compound concentrations rather than just their visible colour in chilli powder. The high linearity of HPLC results with correlation coefficients above 0.998 and recovery in the range of 90.6% to 94.2% show the potential of using the proposed method for quantitative analysis of synthetic dyes. However, laboratory validation of the method should be performed to assess its suitability.

The comparative evaluation of two proposed analytical methods revealed their complementary use in food adulteration analysis. So, UV-Visible spectroscopy is relatively fast, simple and economical technique for preliminary screening of chilli powder samples, but the presence of natural carotenoid pigments and overlapping absorption characteristics may affect its ability to identify the dyes in samples. In turn, HPLC is a better technique for the identification and quantitative estimation due to the separation of individual synthetic dyes. However, the sensitivity of 90.0%, specificity of 80.0% and accuracy of 83.3% obtained for UV-Visible screening show the possibility to use it as a primary technique, along with the necessity for chromatographic confirmation.

To sum up, the results stress the necessity to develop instrumental techniques for the monitoring of synthetic dye adulteration in chilli powder. The combination of UV-Visible spectroscopy and HPLC can be regarded as an effective technique, which combines fast preliminary screening with

identification and estimation of synthetic dyes. Such approach can be helpful for the work of food testing laboratories and quality control agencies.

6. CONCLUSION

The current study investigated an analytical methodology to detect and quantify the presence of synthetic dye adulterants in chilli powder through the use of UV-Visible spectrophotometry and HPLC. The results obtained clearly revealed that both analytical techniques play vital yet distinct roles in the process of food adulteration analysis. Spectroscopy serves as a quick and cost-effective initial screening method while HPLC enables better selectivity in identifying and quantifying individual dyes. As far as the selection of compounds is concerned, Sudan I turned out to be the most frequently detected and high-meaning compound, then comes Sudan IV, Rhodamine B and Metanil Yellow. Thus, the above analysis made it possible to outline the drawbacks of spectroscopic screening and the need for chromatographic confirmation.

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