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A New Simple, Specific and Validated FT-IR Spectroscopic Method for the Estimation of Sudan Dye Adulteration in Chilli Powder

Gokul J Siddharth[1], Fathima A.M[2], Mohammed Shaheer[3], Aleyamma Joseph[4], Saida Sherin[5], Sheeja Velayudhankutty[6]

[1, 2, 3, 4, 5]Research Scholar, Grace College of Pharmacy, Palakkad, Kerala, India

[6]Associate Professor Department of Pharmaceutical Analysis, Grace College of Pharmacy, Palakkad, Kerala, India

[6]Corresponding Author E. Mail: *sheejasureshsree[at]yahoo.com*
Mob: +919995301201

Abstract: ***A simple, rapid, specific and validated method for estimation of Sudan dye II in chilli powder was developed using FTIR. This study was carried out to detect adulterants in chilli powder. Detection test of adulterants which include qualitative and quantitative estimation of Sudan II (carcinogenic dye) in red chili powder by FTIR. For present study twelve chilli powder samples were collected from various localities of Palakkad and analysed. The study includes a detailed investigation. Sudan dye-II and chilli powder sample pellets were prepared by pressed pellet technique. FTIR spectrum obtained with the assistance of the IR solution software, quantitative analysis is carried out in the region 2854cm^{-1} for ring C-H Stretching and 2362cm^{-1} related to N=N. Linearity range was 0.015-0.035mg. Precision was calculated and low %RSD values were found. LOD and LOQ were in the range of 0.0017mg and 0.0054mg. Marketed samples showed the presence of Sudan dye-II whereas homemade sample showed absence of Sudan dye-II. Thus this technique is useful for quality control and detection of powder spices where label free and non branded spices are commonly sold in markets.***

Keywords: Sudan Dye II, Chilli powder, FTIR, Precision, Robustness

1. Introduction:[1,2,3]

Sudan II is a fat soluble azo dye used for staining of triglycerides and some protein bound lipids. It has the appearance of red powder with melting point 156-158^{o}C. In industry it is used to colour non polar substances like fats, waxes, oils, greases, various hydrocarbon products and acrylic emulsions. It was used a food dye under FD & C Red 32 in US until the FDA banned its use in food due to toxicity. IUPAC name for Sudan II is 1-(2,4-dimethylphenylazo)-2-naphthol.

Chilli is type of vegetable that belongs to the family of *Solanaceae*. It is grown in all countries of Asia, Africa, US and Europe. The most important properties of chilli are its colour and pungency. Chilli powder is blended to variety of processed foods. In some part of the Asian countries chilli powder is adulterated mainly with brick powder, in some cases red oxide and Sudan dyes. These dyes are intentionally used as food adulterants especially in red chilli therefore the authentication of chilli powder must be done in order to assure its quality and safety. Many analytical methods have been proposed for identification and quantification of Sudan dyes in chilli powders, majority of them based on chromatographic and spectroscopic procedures. Most of the methods involve complex separation techniques and chemicals that are harmful for the environment.

In this research work sudan-II from samples of chilli powder which were collected from different localities of palakkad town were identified and estimated by convenient and simple FT-IR method. Sudan dye-II and chilli powder sample pellets were prepared by pressed pellet technique.

2. Literature Survey[4,5,6,7,8]

Literature survey reveals there are many chromatographic work, few UV spectrophotometric method and only chemometric FTIR method for the analysis of Sudan dye-II as adulterant. The conventional UV spectrophotometric analysis requires solvent extraction dilution and filtration prior to analysis and generation of results. Even though IR spectroscopy may not as popular as chromatographic method such as HPLC and GC but its non invasiveness, rapidity and simple sample preparation make it valuable analytical method and complement to chromatographic method. The method was developed by FTIR to provide a quick investigation tool for adulterated spices. For present study twelve chilli powder samples were collected from various localities of Palakkad and analysed.

3. Methodology

Chemicals and Reagents

Sudan dye II purchased from Nice Chemicals Pvt.Ltd, Cochin. KBr for FT-IR used was of analytical grade were obtained from S.D.Fine Chemicals (Mumbai, India). All the chilli powder samples purchased from local market of Palakkad.

Instruments:

FT-IR spectrophotometer, Model IR Affinity-1(SHIMADZU), connected to a computer loaded with Shimadzu IR-Solution 2.10 software was used for the measurements. KBr Hydraulic Press used for the pressed pellet technique. The samples were weighed on Shimadzu

balance model AY 220 and Spin spectra 6C centrifuge machine.

Selection of spectral region:
50mg of Sudan dye II was taken and diluted with sufficient amount of potassium bromide to obtain 500mg pellets. From this further dilution was done and transmittance was measured, after obtaining the IR spectrum and with the assistance of IR solution software the quantitative analysis is carried out in the spectral region 2854cm^{-1} and bands had its height analyzed in terms of absorbance.

Obtaining of Analytical curve:
50mg of Sudan dye II was taken diluted with sufficient amount of potassium bromide to obtain 500mg mixture. From this further dilution was done by taking 0.015, 0.02, 0.025, 0.03 and 0.035mg of Sudan II (pure) were taken and diluted with sufficient amount of potassium bromide to obtain 100mg pellets. The powder were mixed and ground until obtaining a homogeneous mixture. Thus, this mixture was compressed in a mechanical die press with 10 ton pressure for 2min to obtain translucent pellets, through which the beam of the spectrometer can pass after obtaining the FT-IR spectrum and with the assistance of the IR solution software. Quantitative analysis was carried out in the spectral region 2854cm^{-1} and bands had its height analyzed in terms of absorbance.

Preparation of Standard Pellets:
50mg of Sudan dye II was taken and diluted with sufficient amount of potassium bromide to obtain 500mg mixture. Sufficient amount of potassium bromide was added to obtain 100mg pellet. Absorbance was measured in the spectral region 2854cm^{-1}

Preparation of Sample Pellets:
For the preparation of stock 10mg of sample was taken and added with sufficient amount of potassium bromide to obtain 100mg pellets. From the stock 2.5mg of sample was weighed and added with sufficient amount of potassium bromide to obtain 100mg pellets. Mixture was compressed in a mechanical die press with 10 ton pressure for 2min to obtain translucent pellets. Quantitative analysis was carried out in the spectral region of 2854cm^{-1} and bands had its height analyzed in terms of absorbance.

Method Validation[9,10,11]
The method was validated by determining the following parameters: linearity, precision, detection limit and quantification limit.

Linearity: Linearity was done with the intension of validate the method, five concentration of standard Sudan dye II 0.015, 0.02, 0.025, 0.03 and 0.035mg were used. Linearity was evaluated by linear regression analysis.

Precision: The precision of the method was evaluated in two requisites: repeatability and intermediate precision. Repeatability (intra-day) was studied by the performance of three determinations of the sample in a concentration 2.5mg per pellet, all in the same day and identical working conditions. Intermediate precision (inter-assay) was assessed by performing the assay in three different days under the same experimental conditions. At the end of test, the percentage relative standard deviation (%RSD) values found to be less than 2.

Detection and quantification limit: The detection (LOD) and quantification (LOQ) limits were calculated based on the intercept standard deviation and the curve slope.

$$LOD=3.3\sigma/S \qquad LOQ=10\sigma/S$$

Where, σ the standard deviation and S is the slope of the curve.

4. Results & Discussion

Estimation of Sudan dye in chilli powder by FT-IR
FTIR was developed for the estimation of Sudan dye-II in chili powder by making pellets of tablet powder by pressed pellet technique. The standard and sample pellets were prepared and spectrums were recorded. The recorded spectrums were given as shown in Table No.1 and Fig.1-13.

Table 1: Estimation of Sudan dye in chilli powder by FT-IR

Sample	Absorbance at (2854cm^{-1})	Amount in Sample (mg)	Remark
Sample-1	2.51	0.073	Present
Sample-2	2.121	0.062	Present
Sample-3	0.20	0.058	Present
Sample-4	1.306	0.038	Present
Sample-5	0.862	0.025	Present
Sample-6	0.771	0.022	Present
Sample-7	0.740	0.021	Present
Sample-8	0.643	0.018	Present
Sample-9	0.39	0.011	Present
Sample-10	0.375	0.0109	Present
Sample-11	0.344	0.010	Present
Sample-12	-	-	Absent

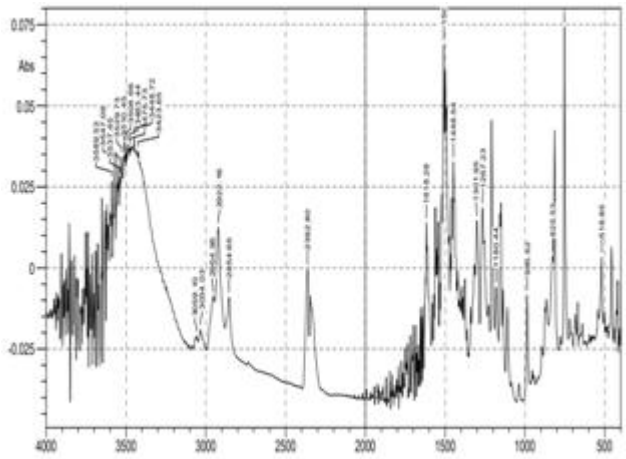

Figure 1: FT-IR Spectrum of standard Sudan II

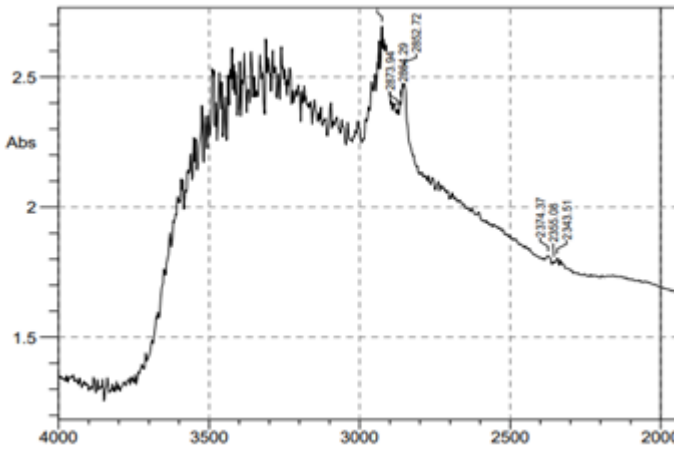

Figure 2: FT-IR Spectrum sample-1

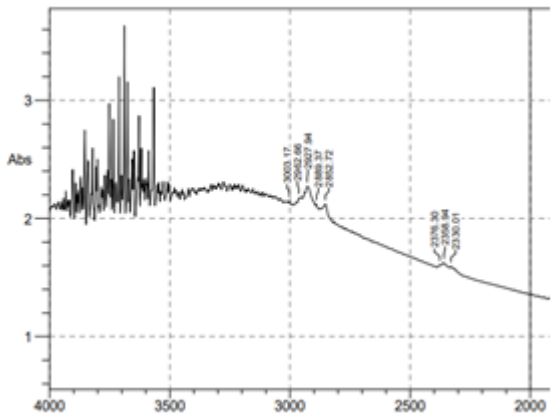

Figure 3: FT-IR Spectrum sample-2

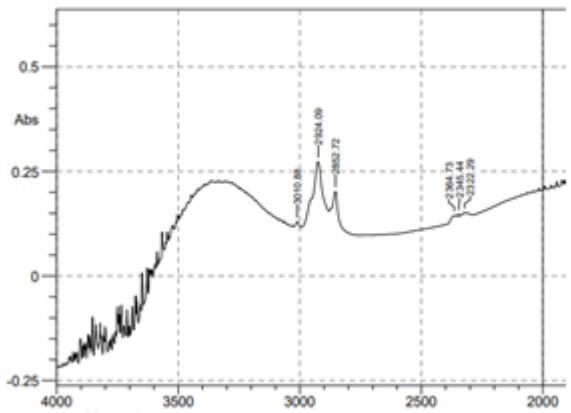

Figure 4: FT-IR Spectrum sample-3

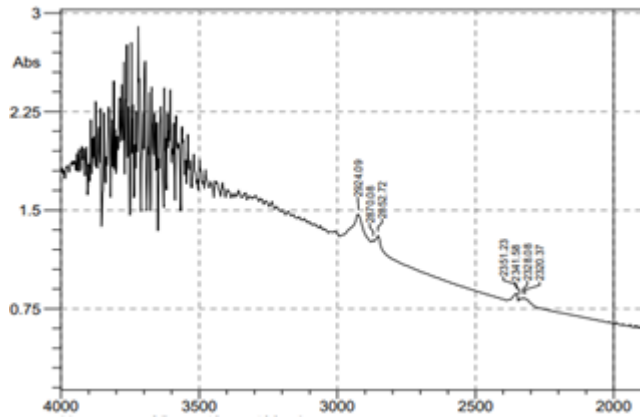

Figure 5: FT-IR Spectrum sample-4

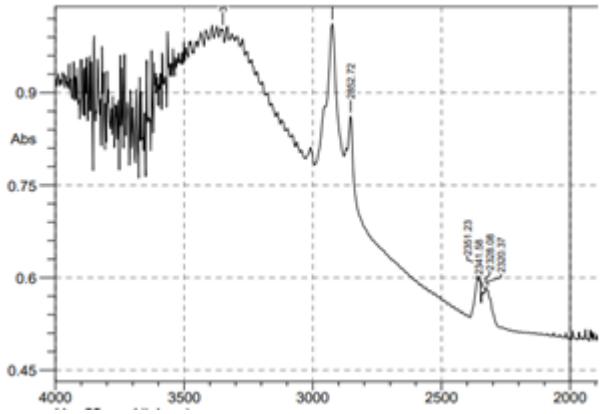

Figure 6: FT-IR Spectrum sample-5

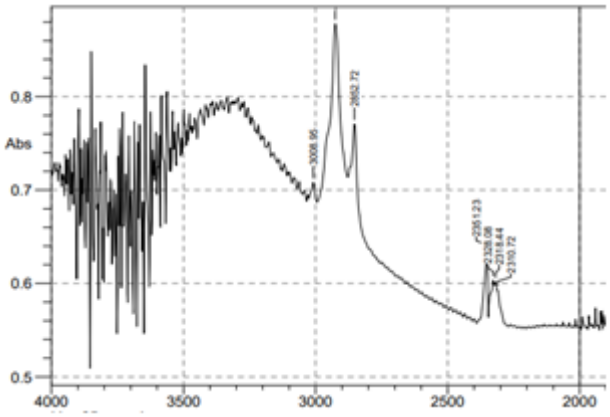

Figure 7: FT-IR Spectrum sample-6

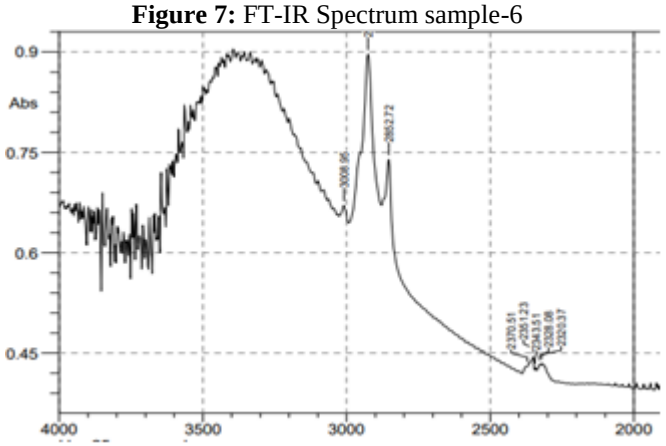

Figure 8: FT-IR Spectrum sample-7

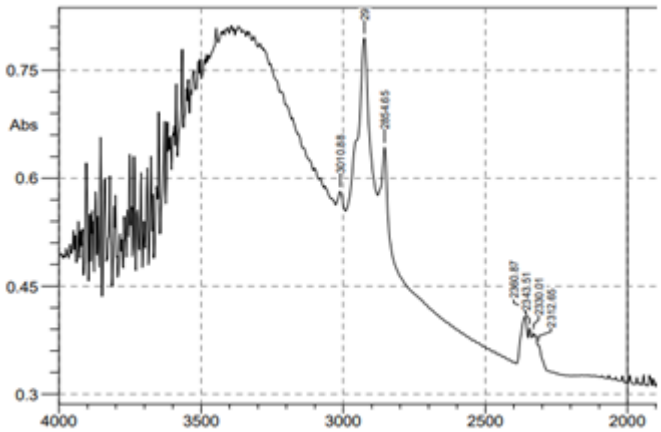

Figure 9: FT-IR Spectrum sample-8

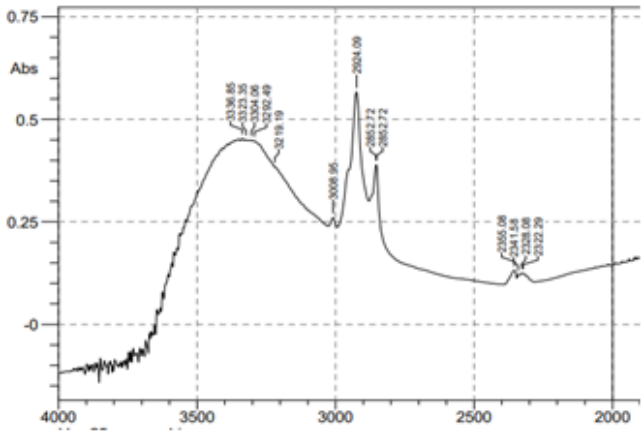

Figure 10: IR Spectrum sample-9

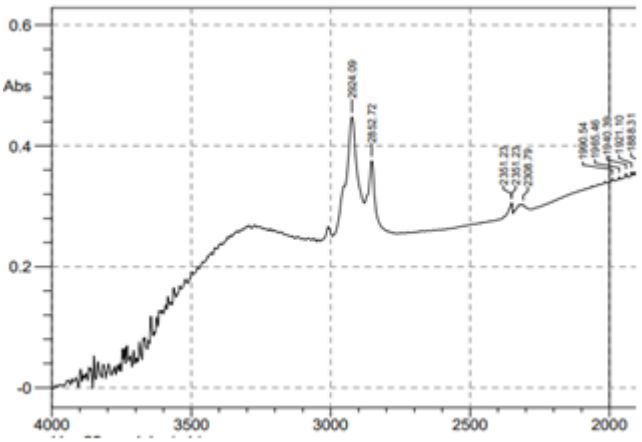

Figure 11: FT-IR Spectrum sample-10

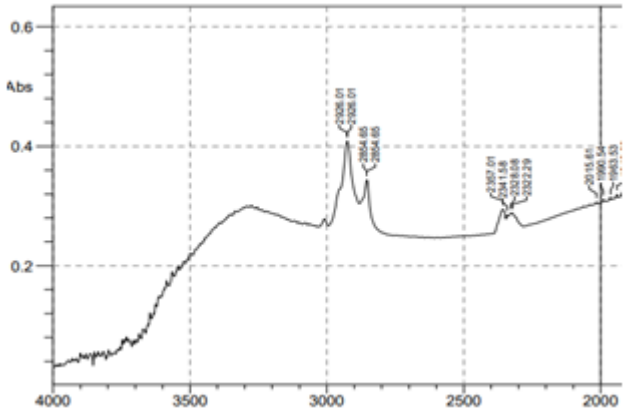

Figure 12: FT-IR Spectrum sample-11

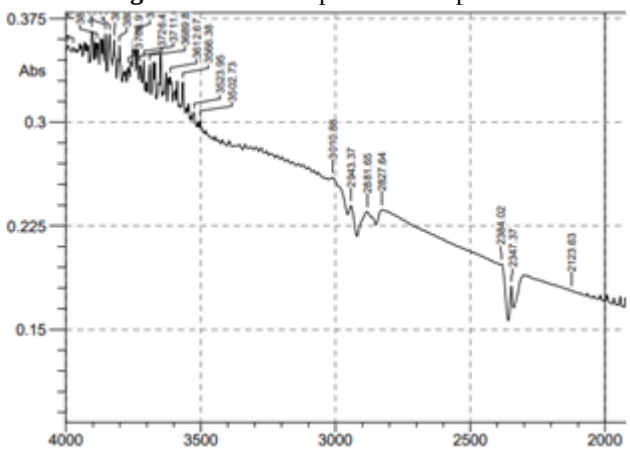

Figure 13: FT-IR Spectrum pure sample

Method Validation

Linearity:

Linearity With the intension of validates the method, five concentration of standard Sudan dye II 0.015, 0.02, 0.025, 0.03 and 0.035mg was used. Linearity was evaluated by linear regression analysis. Absorbances obtained in the above concentration are given in the Table No.2 and Fig.14

Table 2: Linearity for Sudan dye-II

Concentration(mg)	Absorbance
0	0
0.015	0.569
0.02	0.736
0.025	0.893
0.03	1.096
0.035	1.311

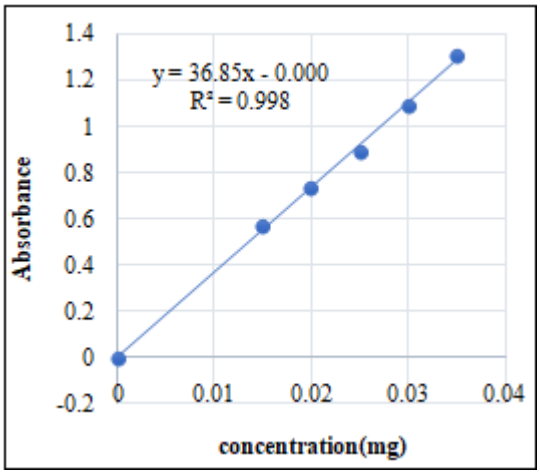

Figure 14: Calibration curve

Precision

The precision of the method was evaluated in two requisites: repeatability and intermediate precision. Repeatability (intra-day) was studied by the performance of three determinations of randomly selected samples in a concentration of 0.025mg per pellet, all in the same day and identical working conditions. Intermediate precision (inter-assay) was assessed by performing the assay in three different days under the same experimental conditions. At the end of test, the percentage relative standard deviation (%RSD) values found to be less than 2 as shown in Table No. 03.

Table 3: Precision results

Sudan dye-II	Concentration (mg)	Sample ID	Intraday n=3		Interday n=3	
			Mean±SD (mg)	%RSD	Mean±SD(mg)	%RSD
	0.025	Sample-1	0.0738±0.006	0.54	0.0734±0.0004	0.54
		Sample-4	0.0378±0.0001	1.07	0.0370±0.0001	0.41
		Sample-8	0.018±0.0001	0.85	0.0180±0.0002	1.15

Detection and quantification limit

The detection and quantification limits were calculated based on the intercept, standard deviation and the curve slope. Results were tabulated in Table No. 04.

$$LOD=3.3\sigma/S \qquad LOQ=10\sigma/S$$

Where, σ the standard deviation and S is the slope of the curve.

Table 4: LOD and LOQ results

LOD (mg)	LOQ (mg)
0.0017	0.0054

5. Conclusion

In FT-IR method for the identification and determination of the Sudan dye-II, KBr pellets containing known amount of standards and samples were used for acquisition of the FTIR spectra. The method involves the measurements of peak of C-H Stretching symmetric (CH_3) at 2854cm-1. Linearity was observed in the range of 0.015-0.035mg for Sudan dye-II. The regression equation for the calibration data was y = 36.853x - 0.0003 with correlation coefficient of 0.9985 for Sudan dye-II. The limits of detection were 0.0017mg and the limit of quantitation was 0.0054mg. The precision of the method was good. The values of the relative standard deviation did not exceed 2%. The proposed method was successfully applied for the estimation of Sudan dye-II. The developed method was found to be precise for the estimation of Sudan dye-II in its pure and various samples of chilli powder.

It can be concluded that the presented method has potential to be used for Sudan dye II in chilli powder due to its rapidness, simplicity, reliability and sensitivity. The major advantages of FTIR method are the following method are sensitive and non destructive or only slightly damage the sample, they require minimal sample preparation and small sample quantities for determination compared to chromatographic procedure and UV spectrophotometric analysis. Spectral analysis by FTIR is simple and don't require laborious sample preparation processes. For the present study, twelve samples of chilli powder were selected out of which eleven marketed samples detected with Sudan II.

6. Future Scope

In this research work classical instrumental spectrophotometric method is described and used for the identification and estimation of Sudan dye II. The method seems to be adequate for the detection of banned Sudan dye II. To assure the validity of results obtained limit of detection and limit of quantification were also determined. The EU announced a decision (2003/460/EC) that specified the limit of detection for Sudan dyes and other similar dyes in the range of 0.5-1.0ppm. Food items containing these dyes above this limit must be discarded. There are additional assets associated with the proposed method such as requires only minute amount of sample (mg), features low cost (consumption of organic solvent is not required) and no special equipment is required for sample treatment. So the method can be applied in routine analysis in labs without extra investments.

So for present study twelve chilli powder samples were collected from various localities of Palakkad and analysed. Hence an attempt was made to develop and validate for the qualitative and quantitative estimation of Sudan dye-II in its pure and as an adulterant in various samples of chilli powder by using Fourier Transform Infrared spectroscopic method (FTIR).

It is an ideal method for identification and estimation of Sudan dyes and has potential application in food control analysis or when screening for banned additives. It is clear from the results of this study that adulterated chilli powders (spices) present a great impact both directly and indirectly on global public health and remain serious problem.

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Author Profile

Sheeja Velayudhankutty, Associate Professor, Dept. of Pharmaceutical Analysis, Grace College of Pharmacy, Palakkad

Gokul J Siddharth, Research Scholar, Dept. of Pharmaceutical Analysis, Grace College of Pharmacy, Palakkad

Fathima A.M, Research Scholar, Dept. of Pharmaceutical Analysis, Grace College of Pharmacy, Palakkad

Mohammed Shaheer, Research Scholar, Dept. of Pharmaceutical Analysis, Grace College of Pharmacy, Palakkad

Aleyamma Joseph, Research Scholar, Dept. of Pharmaceutical Analysis, Grace College of Pharmacy, Palakkad

Saida Sherin, Research Scholar, Dept. of Pharmaceutical Analysis, Grace College of Pharmacy, Palakkad