

# Detection of Adulterants in Red Chili Powder with Special Emphasis on Qualitative and Quantitative Estimation of Sudan I Dye In Red Chili Powder

Margi Gandhi<sup>1</sup>, Rajashree Mashru<sup>2</sup>

<sup>1</sup>Faculty of Pharmacy, Kalabhavan, The M.S. University of Baroda, Baroda, India

<sup>2</sup>Faculty of Pharmacy, The M.S. University of Baroda, India

Corresponding Author: Rajashree Mashru

## ABSTRACT

**Objective:** This study was carried out to detect adulterants in red chili powder.

**Method:** Detection test of adulterants which include presence or absence of brick powder, artificial color, oil soluble color with qualitative and quantitative estimation of Sudan I (carcinogenic dye) in red chili powder.

**Results:** Ten samples were tested out of which none of the sample showed presence of brick powder, seven samples showed presence of artificial colors, six sample showed presence of oil soluble color, six samples was detected by Thin layer chromatography for presence of Sudan I dye and when quantified spectrophotometrically were found in concentration range of 0.3-2.827 ppm.

**Conclusion:** Sudan dyes are very harmful colors that possess carcinogenic effects on human health and their usage should be checked and banned in spices. Thus reliable method was developed to detect presence of Sudan dye in chili powder by both qualitative and quantitative manner.

**Keywords:** Adulterants, Sudan I dye, Red chili powder.

## INTRODUCTION

All humans need food to survive; it is basic necessity of life. A major concern of human societies has been the attainment of sufficient quantities of food to provide to their citizens. When a non permitted substance is added to increase the quantity and quality of the substance then it is termed under adulteration. [1] long back in 1500 BC humans used color additives of natural origin which was safe. But these additives were costly; to reduce cost many synthetic colors were added as color additives.

The synthetic industrial dyes, Sudan dyes belong to group of azo dyes. [2] They were classified into four categories based on their structure: Sudan I [1-(phenyldiazenyl) naphthalene-2-ol], Sudan II [1-(2, 4-

dimethylphenylazo)-2-naphthol), Sudan III [1-(4-(phenyldiazenyl) phenyl) azonaphthalen-2-ol], Sudan IV [1-[[2-methyl-4-[(2methylphenyl) diazenyl]phenyl] naphthalen-2-ol]. Among these Sudan groups of dyes lipophilic Sudan I carcinogenic action was first discovered in 1970's. [2, 3] These azo dyes when eaten spilt into amines which are carcinogenic., Thus they are banned to be added in foodstuff. [4, 5]

The International Agency for Research on Cancer (IARC) has classified Sudan dyes as category three carcinogens and as use of these dyes has major economic consequences for worldwide food industries as well as an impact on public health, they are nonauthorized to be used in the food

industry. [2,6] Yet manufacturer adulterates various spices like red chili powder with these dyes causing severe danger to public health; therefore simple, rapid and reliable analytical methods for the determination of these banned dyes in food stuffs are required. [2] Several techniques have been reported in the literatures which include: Near infrared (NIR) spectroscopy, vibrational spectroscopy, Raman, spectroscopy, [7] Capillary electrophoresis and gas chromatographic methods, [8] High performance liquid chromatography with optical or mass spectrometric detection, [9] HPLC-UV-VIS, [10] Spectrophotometry, [2] Thin layer chromatography [4] In this research work 10 samples of red chili powder were examined for presence of adulterants with special emphasis on Sudan I dye qualitatively and quantitatively.

## EXPERIMENTAL PART

### APPARTAUS AND SOFTWARE

Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UV-Probe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-800 nm. The samples were weighed on electronic analytical balance (A×120, Shimadzu).

### MATERIALS

Standard sample of 1-[(2,4dimethyl phenyl) azo]-2-naphthalenol (Sudan I) dye was purchased from SIGMA ALDRICH. All chemicals used in the present study were of

analytical grade which include Chloroform, methanol, n-hexane, ethyl acetate, sulphuric acid,.

### SAMPLING

Ten samples of chili powder were purchased from local market.

### DETECTION OF BRICK POWDER IN CHILI POWDER [11]

Procedure: Take chloroform/carbon tetrachloride mixture in a beaker. Pour the sample (chili powder) in a beaker containing mixture of chloroform and carbon tetrachloride .Observe the bottom of the beaker. Brick powder and dirt will settle at the bottom if present.

### DETECTION OF ARTIFICIAL COLORS IN CHILI POWDER [11]

Extract the spice sample with petroleum ether. Add 13N Sulphuric acid solution (88 ml of conc. Sulphuric acid diluted with 250 ml of dist. Water). Appearance of Red color that persists even upon adding Dist. Water indicates the presence of artificial color. If the Red color completely disappears on adding Dist. Water, the sample is free of Color adulteration.

### DETECTION OF OIL SOLUBLE COLORS IN CHILI POWDER [11]

Take 2g of the sample in a test tube, add few ml of ether and shake. Decant ether layer into a test tube containing 2 ml of dilute HCL (50%) and shake. Observe color of the lower acid layer. Red color of the lower acid layer indicates the presence of oil soluble color.

Table 1

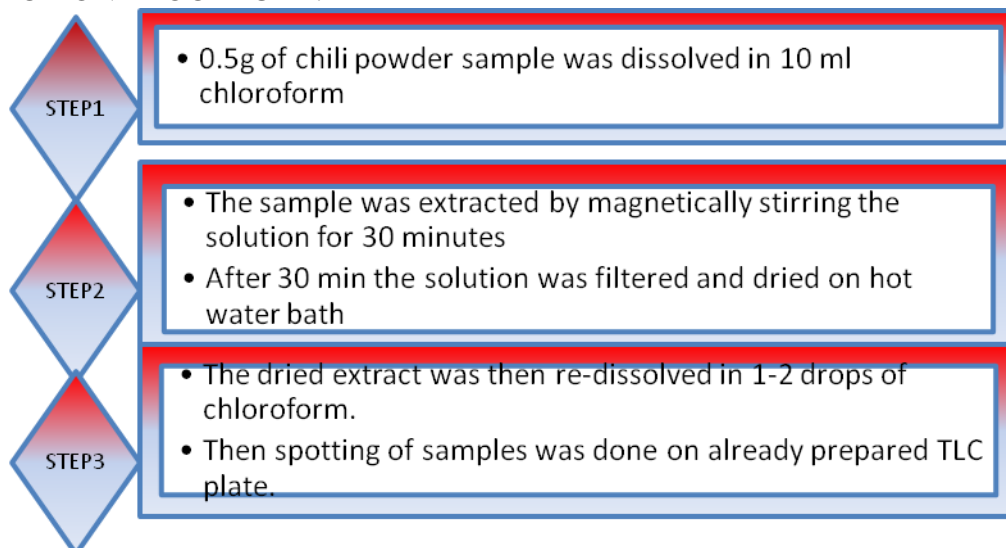
Sample No.	Presence/Absence of brick powder in chili powder.	Presence/Absence of artificial colors in chili powder.	Presence/Absence of oil soluble colors in chili powder.
1	Absent	Present	Present
2	Absent	Absent	Absent
3	Absent	Present	Present
4	Absent	Present	Present
5	Absent	Absent	Absent
6	Absent	Present	Absent
7	Absent	Present	Present
8	Absent	Present	Present
9	Absent	Absent	Absent
10	Absent	Present	Present

## QUALITATIVE ESTIMATION OF SUDAN I IN RED CHILI POWDER

Preparation of standard solution

Stock solutions of standard Sudan red I was prepared by dissolving 1 mg/10 ml of solvent (chloroform) . The working standard solutions were obtained from stock solutions by dilution (1:10) with the solvent.

EXTRACTION PROCEDURE: [4]



## MOBILE PHASE SELECTION

On the basis of the polarity of Sudan I various trails were conducted for optimization of mobile phase shown in Table 2.

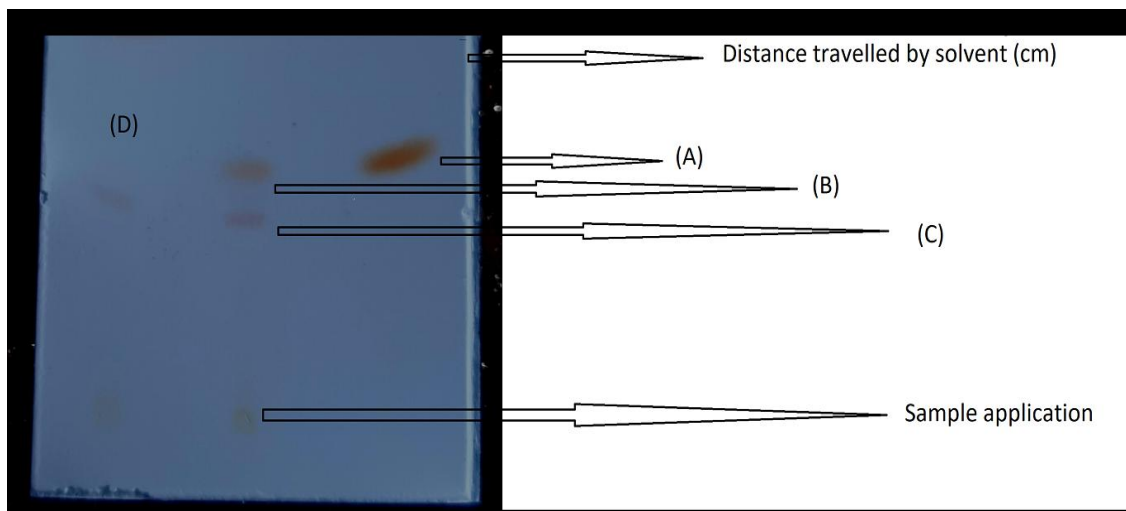
**Table 2**

Mobile phase	Retention factor	Results
Chloroform: Methanol (19:1)	1	Too much polarity, the spot ran with the solvent.
Chloroform: Methanol (18:2)	0.96	Too much polarity, the spot ran with the solvent.
N-hexane: Ethyl acetate (18:2)	0.81	Standard and sample Spot was detected easily. This mobile phase was selected for further qualitative estimation.

The RF values of 10 samples are shown in TABLE 3 showing presence and absence of Sudan I dye in red chili powder.

**Table 3**

Sample no.	Distance Travelled by Solvent (cm)	Mean distance Travelled by Spot (cm)	Absence or Presence of Sudan dyes	RF values
Sample no 1	5.5	4.5	Present	0.81
Sample no 2	5.5	-	Absent	-
Sample no 3	5.5	4.4	Present	0.8
Sample no 4	5.5	4.5	Present	0.81
Sample no 5	5.5	-	Absent	-
Sample no 6	5.5	-	Absent	-
Sample no 7	5.5	4.4	Present	0.8
Sample no 8	5.5	4.6	Present	0.83
Sample no 9	5.5	-	Absent	-
Sample no 10	5.5	4.5	Present	0.81



**Figure 1:** shows representation of TLC plate showing orange spot of standard Sudan I (A), Sample no 1(b) showing orange spot indicating presence of Sudan I in chili powder, 1(c) showing red spot of capsaicin which is main ingredient of chili, sample no 2(d) showing absence of orange spot thus indicating absence of Sudan I in chili powder.

## QUANTITATIVE ESTIMATION

### Standard addition method [2]

In the present work, Standard addition method is used for the spectrophotometric determination of Sudan dyes. Standard addition method can be applied to most analytical techniques and generally use to solve the matrix effect problems. It includes single point standard addition method and Multiple Point Standard Addition method. Here multiple point standard addition method is performed. [2]

### Multiple point standard addition method [2]

The method involves the construction of a calibration curve (fig. 2) to determine concentration of an analyte (Sudan dye) in unknown sample by comparison to a set of samples of known concentration (standards).

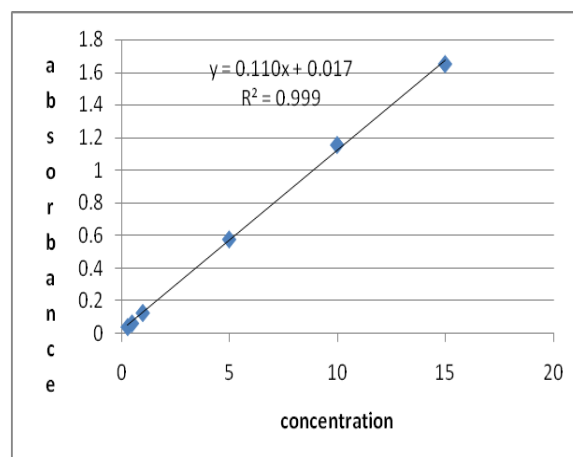
### Preparation of standard (Sudan I) solution

100 ppm stock solutions of standard Sudan red I was prepared by dissolving 1 mg/10 ml of solvent (Ethyl acetate). Further the stock solution was diluted to prepare working

standard solution in concentration range of 0.3-15 ppm.

### Calibration curve

For preparing calibration graph of Sudan I dye, spectra of calibration standards (0.3-15ppm) were recorded in the range of 200 to 800, under the optimum experimental conditions and the absorbance vs. concentration plot was found to be a linear plot as shown in Fig 2. The  $\lambda_{max}$  of Sudan I dye was observed at 471nm. The UV spectrum is shown in Fig 3.



**Figure 2:** linearity plot

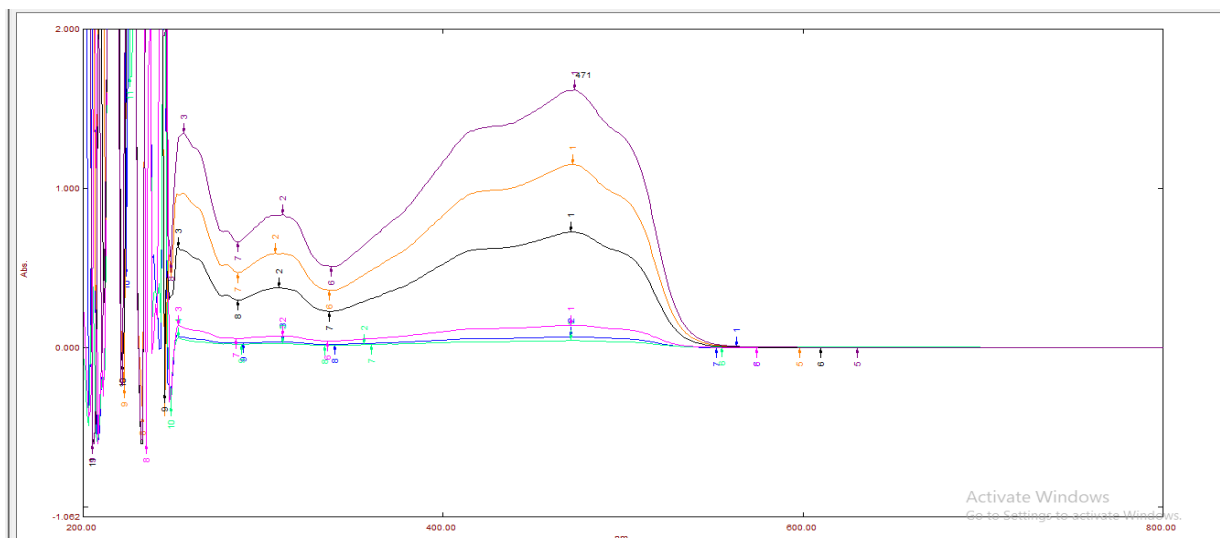
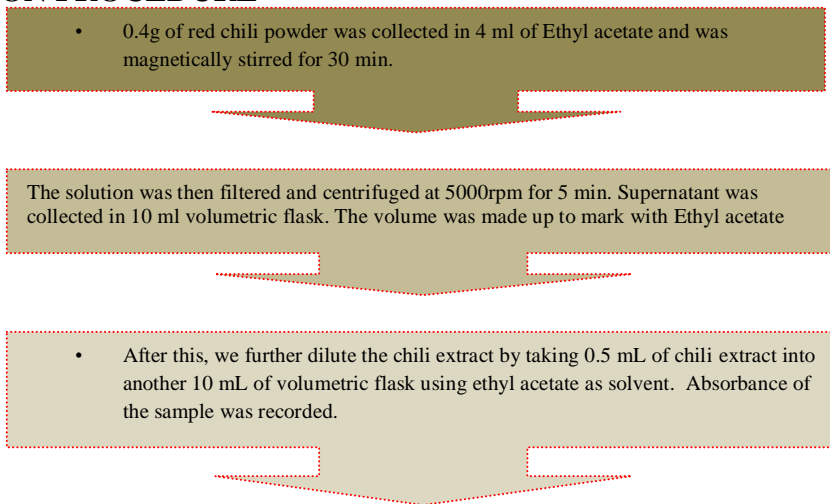


Figure 3: UV spectrum of standard Sudan I in concentration range 0.3-15ppm

### Sample preparation

From qualitative estimation the samples that showed presence of Sudan I in chili powder were analyzed quantitatively.

### EXTRACTION PROCEDURE [2]



### MULTIPLE STANDARD ADDITION METHOD PROCEDURE

Working Standards of Sudan I in concentration range of 0.3-15ppm was prepared from stock solution of 100ppm and 0.5ml of chili sample extract was added into 10 ml volumetric flask using ethyl acetate as make up solvent.

% recovery was also founded by using formula

% recovery= total absorbance- sample absorbance/standard absorbance (from calibration graph). The % recovery was found to be within 95-105%.

SR.NO	Concentration of Sudan I detected in samples (ppm)
Sample 1	2.8
Sample 3	1.6
Sample 4	1.2
Sample 7	1.8
Sample 8	0.6
Sample 10	0.3

### RESULT AND DISCUSSION

Ten samples of chili powder were analyzed out of which six samples showed presence of Sudan I in chili powder. Multiple point standard addition method was preferred because pure chili sample also contain certain types of carotenoids that may also interfere in the analysis in the same wave length regions as Sudan dye

appeared. So, to avoid false positive result and to nullify the affect of matrix multiple point standard addition method is used. The Spectrophotometric method was validated to assure its validity for which limit of detection and limit of quantification was determined. To find LOD and LOQ chili sample which was found unadulterated was manually spiked with Sudan I in concentration range of 0.3-15ppm. A calibration curve was prepared and slope of regression line and standard deviations of the lowest concentration detectable and lowest concentration measured were taken into account. By these values LOD and LOQ were calculated. <sup>[2]</sup> LOD and LOQ were found to be 0.09ppm and 0.27ppm.

#### Authors' Contribution Statement

Dr. Rajashree Mashru has constantly guided this work and Margi Gandhi has prepared the manuscript.

#### CONCLUSION

Sudan dyes are very harmful colors that possess carcinogenic effects on human health and their usage should be checked and banned in spices. They are added in spices to enhance the color of the spices. Thus reliable method TLC and spectrophotometry was developed to detect presence of Sudan dye in chili powder by both qualitative and quantitative manner.

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