# A Method for Simultaneous Detection and Determination of Sudan-I, II, III & IV in Food Stuff by HPLC

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**Summary:** A simple and rapid HPLC method for simultaneous determination of Sudan-I, II, III & IV dyes is described. The method is applied for the determination of dyes Sudan I, II, III & IV in commercial samples of chilli powder, turmeric powder, food color, dry sauce, oil soluble colour and pickles procured from the local market. Limit of detection of method was investigated and found to be 5 ppm.

## Introduction

Sudan-I, II, III & IV are synthetic oil soluble mono azo dye stuff which are genotoxic carcinogens and not permitted in food [1]. These dyes are used in the chemical industry to color oils, waxes, floor waxes and soaps. They are insoluble in water but soluble in organic solvents. These dyes are detected by TLC or UV method after extraction with solvent [2-3].

Separation of Sudan I from interfering substances such as capsanthin, a carotenoid found naturally in chilli, is difficult because it has an absorption maxima at 483 nm, which overlap with the absorption maxima of Sudan I (478 nm). Therefore UV method is not appropriate for differentiation of the two compounds. Qualitative identification method can be performed by TLC; however, for quantitative trace analysis HPLC method is required. In this paper simple and fast method for simultaneous determination of Sudan I, II, III & IV by HPLC has been reported.

## Results and Discussion

First step is to acquire and screen unknown material from commercially available samples. The numbers of samples used in this study were sixteen. These samples were selected at random. These were extracted with acetonitrile following the general procedure given below. Extraction recovery was verified by spiking the sample with 0.01 mg pure Sudan I, II, III & IV successively. Recoveries were found as follows: Sudan I – 90%, Sudan II – 95 %, Sudan III- 85 % and Sudan IV – 92 %.

*Analysis of Standard Mixture of Sudan-I, II, III & IV*

A mixture of Sudan I, II, III & IV in concentration of 5 µg/ml each was prepared. Mobile phase selected was a mixture of four parts of acetonitrile and one part of 0.05 mol/lit methanol containing ammonium acetate and 1ml triethylamine per liter. Flow rate was maintained at 2 ml/min. The detector was set at 478 nm, 20 µl of the solution injected with Rheodyne injector and separated on a Spheri 5, RP-18, 5µm column. HPLC conditions were set such that the analysis of the mixture of standards could accomplish separation of four components. The retention time were found to be 2.05, 2.50, 2.87 and 3.71 for Sudan-I, II, III & IV respectively. The peak areas of the four compounds were found to be almost same.

*Analysis of the Samples*

20 µl filtered acetonitrile extract of chilli powder 1 was injected in the column. The flow rate of mobile phase was maintained 2 ml/min and a peak at 2.05 RT was obtained which showed the presence of Sudan I. The amount of the Sudan I present in the chilli powder was quantified from the area of peak which is found to be 1000 ppm (w/w). Similarly seven samples were analyzed and amount of Sudan-I present were calculated from respective peak areas (Table-1). The samples 2, 5 and 7 having oil soluble colours, after extracting into acetonitrile were analyzed on similar HPLC conditions and gave peaks at 3.71 RT, which shows the presence of Sudan IV.

## Experimental

*Material &Method*

- Commercial samples of chilli powder, turmeric powder, dry sauce, oil soluble color and food color were purchased from the market at random selection.
- Acetonitrile HPLC grade (Merck)

Table-1: Analysis of Sudan I, II, III & IV in Samples

| Sample No. | Name of Sample | Concentration of Sudan | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| 1. | Red chilli powder | + ve (1000 ppm) | - ve | - ve | - ve |
| 2. | Oil soluble color | - ve | - ve | - ve | + ve |
| 3. | Red chilli powder | - ve | - ve | - ve | - ve |
| 4. | Red chilli powder | + ve (100 ppm) | - ve | - ve | - ve |
| 5. | Oil soluble red color | - ve | - ve | - ve | + ve |
| 6. | Dry Sauce | + ve (80 ppm) | - ve | - ve | - ve |
| 7. | Oil soluble red color | - ve | - ve | - ve | + ve |
| 8. | Red chilli powder | + ve (74 ppm) | - ve | - ve | - ve |
| 9. | Red chilli powder | + ve (100 ppm) | - ve | - ve | - ve |
| 10. | Red chilli powder | + ve (200 ppm) | - ve | - ve | - ve |
| 11. | Red chilli powder | + ve (100 ppm) | - ve | - ve | - ve |
| 12. | Red chilli powder | - ve | - ve | - ve | - ve |
| 13. | Red Chilli powder | - ve | - ve | - ve | - ve |
| 14. | Pickle | - ve | - ve | - ve | - ve |
| 15. | Food Color Red | + ve (100 ppm) | - ve | - ve | - ve |
| 16. | Turmeric powder | - ve | - ve | - ve | - ve |

- Sudan-I, II, III & IV (Sigma Aldrich)
- Methanol, Ammonium acetate, Triethylamine
- Water (deionized)
- Filter membrane (0.45 µm)

*Chromatographic Procedure*

The constant pressure high speed liquid chromatograph (Perkin Elmer Norwalk Cmm, USA) (Model-200) is used in this study equipped with a Rheodyne injector and tunable UV absorbance detector. The column used was Spheri-5, Rp-18, 5 µm particle size (250 x 4.6 mm) stainless steel, dry packed and fitted horizontal. Mobile phase was 4 parts of acetonitrile and one part of methanol containing 0.05 mol ammonium acetate and 1 ml triethylamine per litre prepared. The mobile phase was filtered through 0.45 µm membrane filter and degassed by applying degasser (Knauer). Flow rate was 2 ml/min (at 250 psi) 20 µl of extracted sample in acetonitrile were injected with Rheodyne injector on the column with a 25 µl syringe, using a stop flow injection technique.

*Preparation of standard solutions*

50 mg of each Sudan I, II, III & IV accurately weigh were dissolved in acetonitrile (HPLC grade) in a 100 ml volumetric flask and brought to the volume with acetonitrile. An aliquot of this solution was further diluted with acetronitrile to produce a final solution of the desired concentrations.

*Extraction*

10 ± 0.1 g sample of chilli powder was accurately weighed and transferred into 250 ml conical flask and 50 ml acetonitrile were added. The flask was placed in a mechanical shaker for 30 minutes. Supernatant was filtered through filter membrane into a glass vial. This solution was directly used for analysis.

*Calculation*

The amount of Sudan I, II, III & IV were determined from the following expression.

$$\text{Conc. of dye (mg / kg)} = \frac{A}{B} \times \frac{Y}{W} \times D \times 50$$

A = mean area of any identified peak in the test portion extract

B = mean area of peak of interest in the working standard solution

Y = conc. (mg / L) of the suitable working standard solution

W = weight of sample

D = the dilution factor (if any)

**Conclusions**

A simple method for simultaneous determination of Sudan I, II, III & IV is developed. By applying this method level of Sudan in chillies and spices at 5 ppm may be detected.

**References**

1. C. E. Searle, Ed. Chemical Carcinogenesis, ACS Monograph, *American Chem. Soc.*, Washington, D.C. (1976).
2. Official Methods of Analysis of the Association of Official Analytical Chemists Edited by William Horwitz 11th edition, pp 575 (1970).
3. The Analytical Chemistry of Synthesis Dyes edited by Venkataramn, India, pp. 40 (1977).