Spectrophotometric Determination of Sudan I-IV Dyes in Selected Chili Samples: A Survey of Karachi City, Pakistan

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Abstract:

   Sudan dyes are a family of lipophilic azo dyes, extensively used in industrial and scientific applications but banned for use as food colorants due to their carcinogenicity. During recent years, due to the continuing illicit use of Sudan dyes as food colorants their determination in different chili powder and related products has achieved great attention all over the world.
In the present study twelve grinded chili powder samples are collected from various localities of Karachi city and analyzed. There are a number of methods available for the estimation of Sudan I-IV dyes but we used a simple, convenient, analytical and expeditious spectrophotometric method. A simple extraction procedure using ethyl

acetate has been applied for the extraction of these dyes from samples of chili powder. The determination of Sudan I-IV dyes is obtained from chili powder samples by single point standard addition method and multiple point standard addition method.

Key words: *Dyes, Sudan, Standard Addition Method, Spectrophotometer, Karachi*

Introduction

Color is the fundamental and indispensable property for the most chemical industries to enhance their manufacture. Mostly in foodstuff and pharmaceutical industries; natural and synthetic dyes are extensively used. It is the main component of food and almost certainly characteristic facts for appealing human senses (Christie 2001).

Food dye is any substance that is added to food or drink to alter its color, flavor and aesthetic value. Human being has been using a variety of color additives for a long time since 1500 BC and most of those colors were of natural origin. But since 1800 BC with advent of synthetic food dyes, many food industries started using synthetic food dyes without knowledge of their safety. Today, the synthetic food colors are being preferred to natural food colors due to their low cost and tinctorial power (Babu & Shenoliker 1995).

Sudan dyes are synthetic industrial dyes belongs the class of azo dyes. Among them Synthetic Sudan I (fig. 1a), Sudan II (fig.1b.), Sudan III (fig. 1c) and Sudan IV (fig. 1d) are widely used as coloring agent in fuels, spirit vanishing, plastics, shoe and floor polishes, waxes, printing inks and in the leather and fabric industries (Dillon et al. 1994; Rafi et al. 1997). They have been used in scientific research and cosmetic products (Cornet et al. 2006). Scientific applications include staining of lipids with Sudan IV for visualization and analytical purpose (Chen et al. 2002). For many years Sudan dyes have been used as additive in food products by some factories because of their intensive red-orange color. Among the Sudan group of dyes

lipophilic Sudan I (fig. 1a.) carcinogenic action was first discovered in 1970's (Abraham et al. 2002).

In April 2003, the sudden I was detected in hot chili and hot chili products of India and spread fears European Union (EU). And two years later, in March 2005, similar food panic happened in china as well as in the EU again. In china, Sudan I was found in many food products from the international famous brands, such as in chili oil, baking chicken, spicy turnip pickle and chili powder (Daood & Biacs 2005; Sproll et al. 2005).

In 2005 the European Food Safety Authority (EFSA) initiated a review of the toxicology of a number of dyes found illegally in food in the EU. The (EFSA) came to the conclusion that, especially for Sudan I, there is strong evidence for both genotoxicity and carcinogenicity. Because of structural similarities between Sudan I and the other Sudan dyes, the larger group is presumed to have the same deleterious effects (EFSA 2005).

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 non-authorized to be used in the food industry (IARC 2008).
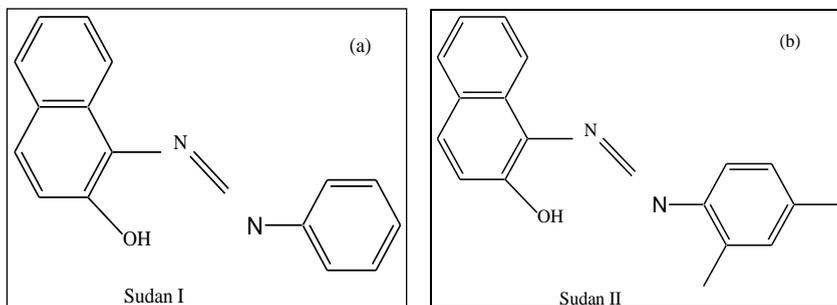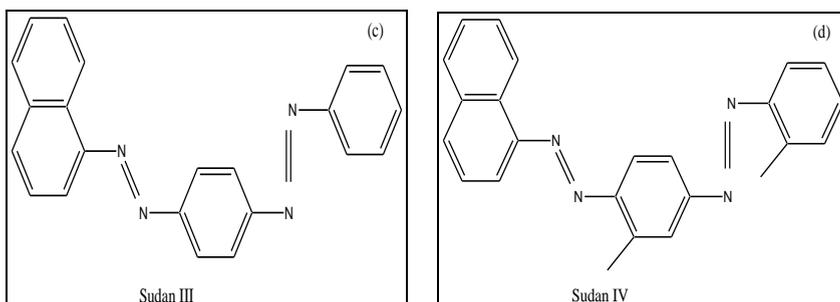
Fig. 1. Structure of Sudan I, Sudan II, Sudan III and Sudan IV

The illegal use of Sudan dye is a severe danger to public health; therefore simple, rapid and reliable analytical methods for the determination of these banned dyes in food stuffs are required. Several techniques have been reported in the literatures and mostly proposed methods are based on high performance liquid chromatography, with optical or mass spectrometric detection (Di Donna et al. 2004; Botek et al. 2007; Capitan et al. 1996). However, some gas chromatographic and capillary electrophoresis methods have also been reported (He et al. 2007). Generally, chromatographic methods are expensive and times consuming therefore, other techniques for detecting Sudan dyes have been developed (Han et al. 2007; Mustafa et al. 2013).

In this research work, Sudan dyes (I-IV) from samples of chili powder which are collected from different localities of Karachi city are extracted and estimated by convenient and simple spectrophotometric method. Ethyl Acetate has been used for the extraction of these dyes from samples of chili powder. Sudan dyes (I-IV) are first determined by single point standard addition method and then by multiple point standard addition method.

Experimental Work

Materials and Methods

Toluene, ethyl acetate, methanol (Sigma Aldrich), 1-[(2,4-dimethyl phenyl) azo]-2-naphthalenol (Sudan I), 1-(phenyl azo)-2-naphthol (Sudan II), 1-(4 phenyl azo phenyl azo)-2- naphthol (Sudan

III), o-tolyl azo-O-tolyl azo-beta-naphthol (Sudan IV), silica gel TLC card (Merck), Iodine reagent, UV-Vis Lamp, Jenway 6310 Spectrophotometer, Analytical balance, Select spin spectra 6C centrifuge machine, Fluko homogenizer and 0.45 µm filter membrane.

Sampling

Twelve samples of chili powder have been collected from different localities of Karachi city during January 2013 to March 2013.

Standard Addition Method

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. In the method two different statistical approaches that is single point and multiple point are adopted.

Single point Standard Addition

The method involves the preparation of only one standard and then the concentration of Sudan dyes I-IV in sample is determined by following equation:

Concentration = $A_u/A_s+A_u \times (C_s + C_u)$

Where,

A_u = Absorbance of Sudan dye in Sample.

A_s = Absorbance of Sudan dye in Standard.

C_s = Concentration of Sudan dye in Standard.

C_u = Concentration of Sudan dye in Sample.

Multiple Point Standard Addition

The method involves the construction of a calibration curve (fig. 3) to determine concentration of an analyte (Sudan dye) in unknown sample by comparison to a set of samples of known concentration (standards).

Extraction and Sample Preparation

Chili powder samples were obtained from the local markets of Karachi city and homogenized properly. 2.5 grams of chili powder samples

were accurately weighed and transferred into a 30 mL centrifuge tube containing 20 mL of ethyl acetate. The mixture was blended with homogenizer at 16,500 rpm for 5 minute and then centrifuge for 5 minute, after centrifugation upper organic layer was filtered and collected in a 25 mL volumetric flask and made up the volume with ethyl acetate. After this, we further dilute the chili extract by taking 0.5 mL of chili extract into another 25 mL of volumetric flask using ethyl acetate as solvent.

Preparation of Standards of Sudan I-IV Dye

Four standards of each Sudan dye in concentration range of 1-50 ppm were prepared by taking 0.1-50 mL of Sudan stock solution of 100 ppm and 0.5 ml of chili sample extract into 25 mL of volumetric flasks using ethyl acetate as make up solvent.

The experimental work is summarized in figure 2.

Visible spectrums of standards were recorded in the wavelength range of 320-750 nm. The 476 nm λ for Sudan I, 494 nm λ for Sudan II, 508 nm λ for Sudan III and 520 nm λ for Sudan IV were selected from the spectra due to their maximum absorbance at the respective wavelengths. Finally the absorbance of each sample was measured for the presence of these dyes at these wavelengths (Table 2).
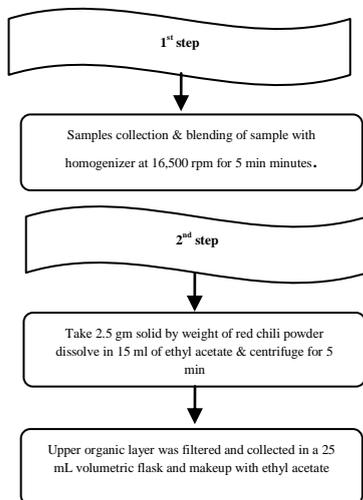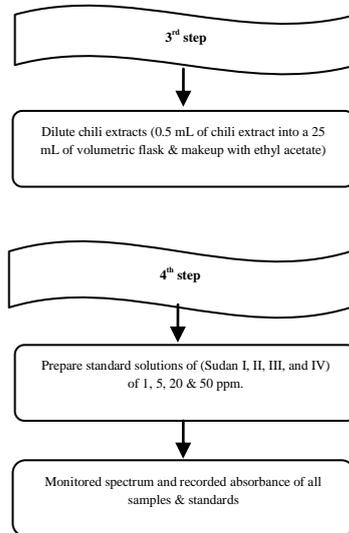
Fig. 2. Block diagram of method used for the estimation of Sudan (I, II, III, and IV) in chili powder samples

Table: 1. Absorbance of standards (1.0-50 ppm of Sudan I to IV) and samples for constructing calibration curve for multiple point standard addition method.

Sampling Area	Conc. (ppm)	Sudan I [b]476 nm	Sudan II [b]494 nm	Sudan III [b]508 nm	Sudan IV [b]520 nm	Sampling Area	[a]Conc. (ppm)	Sudan I [b]476 nm	Sudan II [b]494 nm	Sudan III [b]508 nm	Sudan IV [b]520 nm
Gulshan-2	1	0.084	0.090	0.069	0.053	Q. Abad	1	0.066	0.130	0.141	0.067
	5	0.232	1.136	0.146	0.088		5	0.210	0.354	0.432	0.177
	20	0.718	0.898	0.838	0.386		20	0.835	1.042	1.178	0.575
	50	1.040	0.257	1.208	1.000		50	1.113	1.217	1.291	1.137
	Sample	0.236	1.140	0.149	0.089		Sample	0.213	0.356	0.433	0.179
Landhi	1	0.050	0.098	0.090	0.045	Saddar	1	0.035	0.060	0.026	0.032
	5	0.247	0.279	0.343	0.340		5	0.207	0.274	0.072	0.532
	20	0.699	0.905	0.856	0.420		20	0.789	0.984	0.396	0.877
	50	1.057	1.148	1.215	1.122		50	1.060	1.165	0.923	1.277

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	Sample	0.249	0.276	0.342	1.205		Sample	1.250	0.956	1.590	0.890
Malir	1	0.080	0.130	0.138	0.133	CBA lines	1	0.080	0.117	0.097	0.065
	5	0.238	0.331	0.503	0.432		5	0.228	0.305	0.239	0.085
	20	0.695	0.806	1.199	0.680		20	0.688	1.152	0.983	0.266
	50	1.060	1.194	1.263	1.128		50	1.036	1.134	1.196	1.190
	Sample	0.956	0.450	0.601	0.450		Sample	0.230	0.306	0.986	0.095
Sachal Goth	1	0.124	0.152	0.116	0.132	Joriya bazaar	1	0.039	0.071	0.095	0.034
	5	0.320	0.330	0.508	0.522		5	0.217	0.218	0.380	0.320
	20	0.720	0.961	1.111	0.619		20	0.788	0.783	1.158	0.702
	50	1.075	1.174	1.255	1.108		50	1.098	1.176	1.258	0.973
	Sample	0.325	0.340	0.516	1.059		Sample	1.520	1.210	0.520	0.481
Korangi	1	0.078	0.075	0.097	0.050	Kala Pul	1	0.031	0.080	0.112	0.096
	5	0.196	0.285	0.273	0.130		5	0.107	0.272	0.365	0.350
	20	0.736	0.965	0.751	0.240		20	0.877	0.949	1.095	0.828
	50	1.101	1.198	1.251	0.883		50	1.063	1.150	1.245	1.141
	Sample	1.215	0.520	0.356	0.455		Sample	0.107	1.256	0.658	0.496
Jamshed Road	1	0.039	0.071	0.095	0.034	Gulshan-1	1	0.122	0.120	0.134	0.079
	5	0.217	0.218	0.380	0.049		5	0.274	0.322	0.301	0.167
	20	0.788	0.783	1.158	0.402		20	0.718	0.874	1.074	0.524
	50	1.098	1.176	1.258	0.973		50	1.087	1.194	1.256	1.056
	Sample	0.218	0.219	1.256	0.050		Sample	0.278	0.325	0.301	0.168

Figure: b. Calibration curve for Sudan-II

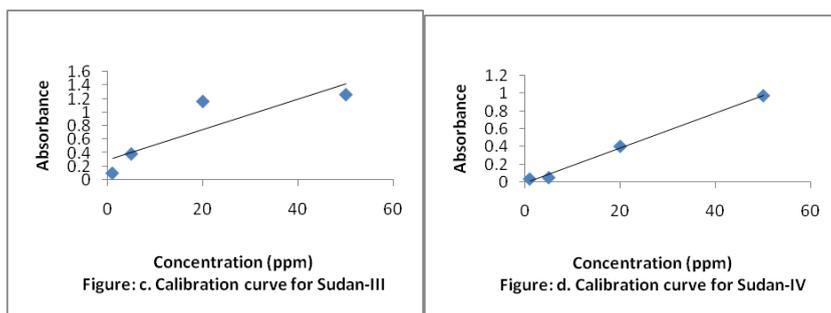Figure: a. Calibration curve for Sudan-I
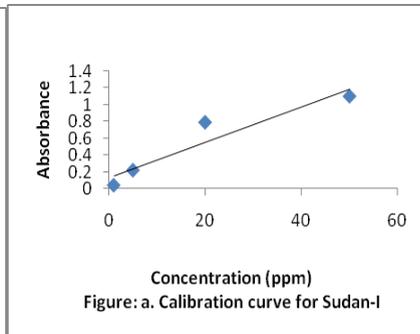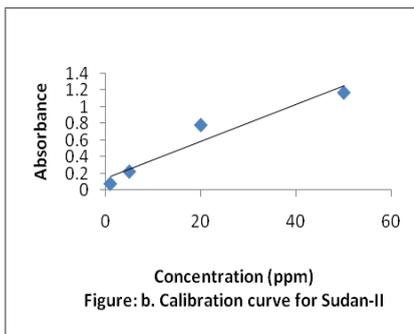
Fig. 3: Calibration curve for Sudan-I to IV for Jamshed road

Result and Discussion

In this research work a conventional classical instrumental spectrophotometric method is described and used for the estimation of Sudan dyes. The Spectrophotometric method seems to be adequate for the detection of banned Sudan dyes (I-IV). To assure the validity of results obtained limit of detection and limit of quantification were also determined. For this purpose pure chili powder sample those did not previously adulterated were manually spiked with 0.01-50 ppm concentrations of Sudan I to IV. A calibration curve was prepared and slop 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. The LOD and LOD values obtained are same that is 0.025 ppm.

Twelve (12) samples of chili powder have been collected from different local areas of Karachi, Pakistan. Two different statistical approaches were adopted for the estimation of dyes by the method developed. One is single point standard addition method and other is multiple point standard addition method (Calibration Curve method) (fig. 3) for spectroscopic analysis. The standard addition method is preferred on external standard method because pure chili sample also contain certain types of caretonides that may also interfere in the analysis in the same wave length regions as Sudan dyes appeared. So, to avoid false positive result and to nullify the affect of matrix standard addition method is used. The results obtained by both

approaches prove the reliability of data because the results obtained by both methods were almost same.

Ethyl acetate was used for extraction of dyes from chili samples and to prepare solutions. The use of Sudan dyes as food additives is very common in our country and it is used in food products as colorant. The dye is very harmful and its use for human consumption is prohibited due to their carcinogenicity (cancer producing capacity), red chili powder containing Sudan dyes are strictly banned and should not be allowed to place in the market. Therefore, we developed a simple, convenient and reliable method to estimate these dyes.

Spectrum of pure dyes standards were also recorded to monitor the wavelength at which the absorbance of each Sudan dye was maximum. Spectrum of Sudan I dye shows λ_{max} at 476 nm, spectrum of Sudan II dye shows λ_{max} at 494 nm, spectrum of Sudan III dye shows λ_{max} at 508 nm and spectrum of Sudan IV dye shows λ_{max} at 520 nm. These wavelengths were used to monitor the concentration of each dye in samples.

Among the twelve collected samples, Malir, Korangi, Saddar and Joriya Bazaar areas chili powder show maximum concentration of Sudan I, II, III, and IV dyes. While, Q. Abad and Gulshan-1 areas shows the absence of Sudan I-IV dyes, Jamshed road area shows the presence of Sudan III dye, Landhi area shows the presence of Sudan IV dye, Gulshan-2 area shows the presence of Sudan II dye, Sachal goth area shows the presence of Sudan IV dye, CBA Lines area shows the presence of Sudan III dye and Kala pul area shows the presence of Sudan II, III and IV dye. The ranges of concentration are 0.184-2.878 ppm (Sudan I), 0.112-3.488 ppm (Sudan II), 0.081-0.948 ppm (Sudan III) and 0.025-3.176 ppm for Sudan IV by single point standard addition method and 0.190-2.300 ppm (Sudan 1), 0.115-3.49 ppm (Sudan II), 0.080-1.00 ppm (Sudan III) and 0.028-3.189 ppm (Sudan IV) by multiple point standard addition method. The over all range of concentration of Sudan dyes determined in samples were 0.025-3.488 ppm by single point standard addition method and 0.028-3.490 ppm by multiple point standard addition method for Sudan IV dye in Sadder sample and for Sudan II dye in Kala pul sample, respectively

(Table-2). The results obtained by multiple point method as compared with single point method are more reliable due to minimization of random error.

Table: 2. Concentrations of Sudan dyes (I-IV) by single point and multiple point standard addition methods

Sampling Area	Sudan	Concentration (ppm)		Remarks	Sampling Area	Sudan	Concentration (ppm)		Remarks
		Single Point Method	Multiple Point Method				Single Point Method	Multiple Point Method	
Gulshan-2	I	-	-	Absent	Q. abad	I	-	-	Absent
	II	-	-	Absent		II	-	-	Absent
	III	-	-	Absent		III	-	-	Absent
	IV	-	-	Absent		IV	-	-	Absent
Landhi	I	-	-	Absent	Saddar	I	0.455	0.500	Present
	II	-	-	Absent		II	0.157	0.160	Present
	III	-	-	Absent		III	0.936	0.9450	Present
	IV	2.826	2.830	Present		IV	0.025	0.028	Present
Malir	I	0.701	0.781	Present	CBA lines	I	-	-	Absent
	II	0.244	0.248	Present		II	-	-	Absent
	III	0.102	0.110	Present		III	0.840	0.842	Present
	IV	0.107	0.110	Present		IV	-	-	Absent
Sachal goth	I	-	-	Absent	Joriya bazaar	I	2.878	2.300	Present
	II	-	-	Absent		II	0.925	0.930	Present
	III	-	-	Absent		III	0.194	0.195	Present
	IV	3.176	3.189	Present		IV	0.125	0.130	Present
Korangi	I	0.184	0.190	Present	Kala pul	I	-	-	Absent
	II	0.112	0.115	Present		II	3.488	3.490	Present
	III	0.081	0.080	Present		III	0.626	0.628	Present
	IV	0.128	0.130	Present		IV	0.187	0.188	Present

Jamshed road	I	-	-	Absent	Gulshan-1	I	-	-	Absent
	II	-	-	Absent		II	-	-	Absent
	III	0.948	1.000	Present		III	-	-	Absent
	IV	-	-	Absent		IV	-	-	Absent

Conclusion

Therefore, it is concluded that the presented spectrophotometric methods can be used for the detection of Sudan dyes in chili powder in ppm level. The method is suitable and reliable for identification and estimation of Sudan I-IV in chili samples and the most of the samples collected from various areas of Karachi are adulterated with the banned dyes Sudan I, II, III and IV.

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