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Estimation of Para Red Dye in Chilli Powder and Tomato Sauces by a Simple Spectrophotmetric Method followed by Thin layer Chromatography

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ABSTRACT: A number of grinded chilli powder samples and tomato sauce samples were collected from various localities of Karachi city. All the samples were estimated for the presence of para red which is an azo dye and more specifically belongs from the group of sudan dyes. According to the regulation of various governmental agencies its use in food is strictly prohibited because of its ability to form carcinogenic compounds. Although there were a number of sophisticated chromatographic methods for the estimation of this dye but we developed here a simple, convenient, expeditious spectrophotometric method followed by thin layer chromatography technique and compared the results by single point and multiple point external standard methods. On the basis of these results we also specify the localities of Karachi city where the contamination is more prominent. © JASEM

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Color is an imperative constituent of foods and most likely the first characteristic perceived by the human senses. All foods from raw agricultural supplies to finished food stuffs have an associated particular color (Babu and Shenolikar, 1995). They always remain a part of our life since stone ages and added in food items to enhance the appetite of person. In ancient time plants are the only source of color but the problem with natural dyes (chlorophylls, carotinoids, flavonoids and anthocyans) is that they easily degraded during processing and storage. Today, there are extensive numbers of synthetic dyes available in markets used not only to enhance colors but also enhance the presentation and acceptability of food products, where no natural colors exist. Unfortunately, many food items manufactuers used these dyes to cover aging effects, to masquerade decay, and/or to disguise poor foodstuffs. Moreover, they even did not hesitate to use textile dyes, which are dangerous to health as food colorants (Khanna and Singh, 1975; Tripathi et al., 2007). Food dyes may give the impression of being tastier, but a major watch dog group says they cause numerous health problems and are trying to draw attention of the government to disallow them. It has been investigated that even not only the degradation products of these dyes but also the synthetic precursors and intermediates could be highly dangerous due to their toxic and carcinogenic nature (Clarke and Anliker, 1980). The majority of dyes could be an origin of allergic reactions, eczema, skin dermatoses (Nikulina et al., 1995), affect the liver (Jaskot and Costa, 1994; Nikulina et al., 1995), the lungs (Ballantyne, 1994), the vascocirculatory system (Przybojewska, 1996), the reproductive system (Eastin et al., 1996; Nikulina et al., 1995) and the immune system (Ng, 1995). While dyes of aromatic structures (Clarke and Anliker, 1980), which contain azo linkage, amino or nitro groups are cancer causing (Dipple and Bigger, 1991) in experimental animals as well as for humans.

A series of high performance liquid chromatography methods have been previously reported in literature. A rapid HPLC method with UV-Vis detector for the determination of sudan and para red dyes in red chilli peppers on a reverse phase C-18 column with isocratic elution, using a mobile phase of acetonitrile/methanol with detection at 506 nm was notified (Ertas et al., 2007). In the same year a reversed phase HPLC coupled with mass spectrometry was appeared in references for the estimation of these dyes in acetonitrile extract acidified with acetic acid (Botek et al., 2007). In continuation of our on-going studies in 2009, a high performance liquid chromatographic method has been reported for the determination of para red dye in chilli powder and other related products (Riaz et al., 2009). However, recently a simultaneous determination of fat soluble synthetic colors sudan I-IV and para red in spices were successfully achieved isocratically in less than 5 min on the narrow bore monolithic column using HCOOH/acetonitrile as mobile phase (Zacharis et al., 2011). Other than the chromatographic method, a number of methods have been developed and validated for the estimation of para red and sudan dyes in various products with satisfactory results, including a heterologous ELISA (enzyme linked immunosorbent assay) to simultaneously determine para red and sudan dyes in egg (Chang et al., 2011) and a spectrophotmetric method involving second order calibration algorithms that based on the second order spectra for Sudan I in chilli samples (Yuan et al., 2008).

This research work will basically emphasis on the determination of azo bond containg para red dye (Fig. 1) in hot chilli powder and tomato sauce samples collected from local market of Karachi city by a simple and economical spectrophotometric method followed by TLC (thin layer chromatography).

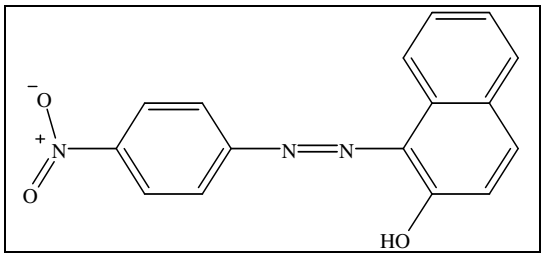

Fig. 1: Structure of para red

MATERIALS AND METHODS: Para red standard (95%, Aldrich), toluene, ethyl acetate, methanol (Sigma Aldrich), Silica gel 60G F254 TLC Card (Merk, Germany), 0.45 µm membrane filter, UV-Visible, JENWAY 6310 Spectrophotometer, Analytical Balance (Denver Instrument, TP-214), Select Spin™ Spectra 6C Centrifuge machine and FLUKO homogenizer were used.

Two different approaches were adopted for analysis of para red dye one is single point external standard method and other is multiple point external standard method.

The single point external standard method involves the preparation of only one standard and later on the concentration of para red in sample is determined by following equation;

Au/As = Cu/Cs

Where

Au = Absorbance of para red in sample

As = Absorbance of para red in standard

Cs = Concentration of para red in standard

Cu = Concentration of para red in sample

The multiple point external standard method involves the preparation of a series of standards as given in Table 1. By making a graph simply named as calibration curve (Fig. 2 & Fig. 3) the concentration of para red in sample is determined by following calibration equation;

y = mx + c

Where

y = Detector response (Absorbance)

m= Slope

x = Concentration

c = Intercept

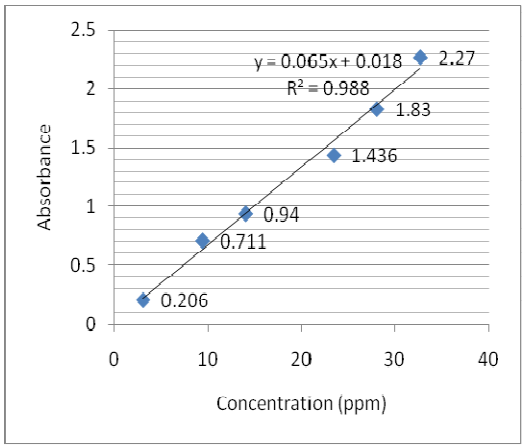

Fig. 2: Calibration standards for the para red dye in toluene 3.07 to 32.7 ppm

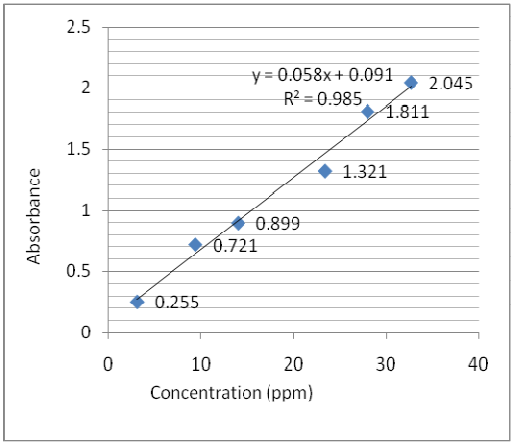

Fig. 3: Calibration standards for the para red dye in ethyl acetate 3.07 to 32.7 ppm

Initially, the solubility of para red standard dye was checked in different solvents and found that it was completely soluble in toluene and ethyl acetate. The stock solutions were prepared in toluene and ethyl acetate of the same concentration 96 and 99 ppm, respectively, in 50 mL volumetric flask and made up the volume up to the mark. Further required dilutions were prepared from these stock standard solutions as shown in Table 1.

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Table 1: Multiple point Method: Calibration standards for chilli powder & tomato sauce samples in toluene and ethyl acetate respectively

S.No.	Conc. of Para Red Standards ppm	Absorbance (in toluene)	Absorbance (in ethyl acetate)
1.	3.07	0.206	0.255
2.	9.36	0.711	0.721
3.	14.0	0.940	0.899
4.	23.4	1.436	1.321
5.	28.0	1.830	1.811
6.	32.7	2.270	2.045
Equation of calibration		y = 0.065x + 0.018	y = 0.058x + 0.091
Correlation coefficient (R^2)		0.9889	0.9851

The chilli powder and tomato sauce samples were obtained from the local market of the Karachi city and homogenized properly. The 5 gram of each sample was accurately weighted into a 50 mL centrifuge tube and 20 mL of toluene was added, then the sample was blended with homogenizer at 6500 rpm for 1 min. The extracts were centrifuged at 4000 rpm for 10 min and the upper organic layer was filtered into a 25 mL volumetric flask and the volume was made up with the same solvent up to the mark. The extract was filtered using 0.45 µm membrane filter. All the filtrate was collected in a 25 mL volumetric flask and the volume is finally made up to 25 mL. Same procedure was adopted for tomato sauces using ethyl acetate as organic solvent for extraction.

Initially retention factor (Rf) values of each sample extract was monitored by TLC with respect to pure para red standard, then the spectra of both standard in toluene and ethyl acetate were recorded in the wavelength range of 380-570 nm (Fig. 4 & 5). The 488 nm as λ_{max} was selected from the spectra and the absorbance of each sample was measured.

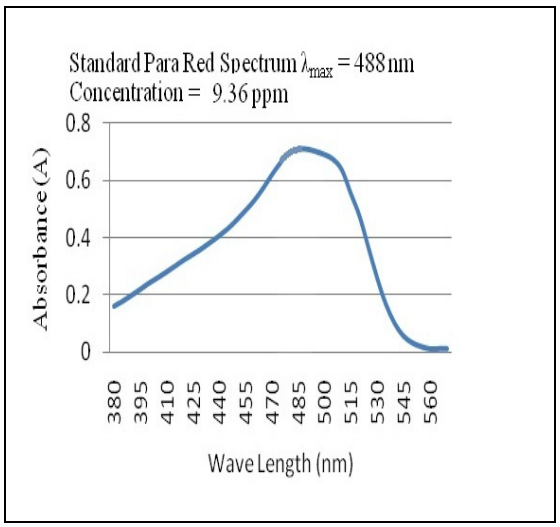

Fig. 4: Spectrum of para red standard dye in toluene

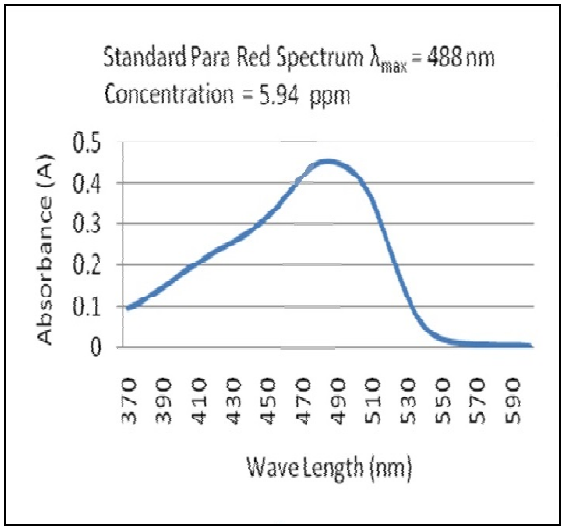

Fig. 5: Spectrum of para red standard dye in ethyl acetate

RESULTS AND DISCUSSION:

The EU announced a decision (2003/460/EC) that specified the limit of detection for sudan I and other similar dyes in the range of 0.5-1.0 ppm. Food products containing these dyes above this limit must be discarded. The decision was amended in January 2004 (2004/92/EC) and included Sudan II, III, and IV. Besides the carcinogenic metabolites of para red, due to highly structural similarities to sudan I, it is assumed that para red is itself potentially genotoxic and possibly carcinogenic and hence taken into account of banned dyes (Brantom, 2005).

In the present study, 40 samples of chilli powder and tomato sauces from various localities of Karachi city have been randomly collected during March-2012 to June-2012 and analyzed by presented method. Over all 16 of those samples were contaminated with para red. The percentage of contamination of para red in chilli samples was more than the tomato sauce samples. Two different statistical approaches were adopted for the estimation of dye in sample that is single point and multiple point external standard methods. Maximum values of 140 ppm were obtained in chilli powder sample of orangi town (OT-2) and 100 ppm in tomato sauce of sadder (SD-1). In chilli samples, the range of concentration for para red was found from 22.3-139.5 ppm by single point method and 24.2-140.0 ppm by multiple point method, whereas in tomato sauces the concentrations were in the ranges of 3.30 to 99.95 ppm by single point method and 3.28-100 ppm by multiple point method as mentioned in Table 2 & 3. The results obtained by multiple point method (Method B) is better than the single point method (Method A) because precision of method B is enhanced due to running series of standards as presented in Fig. 6 & 7.

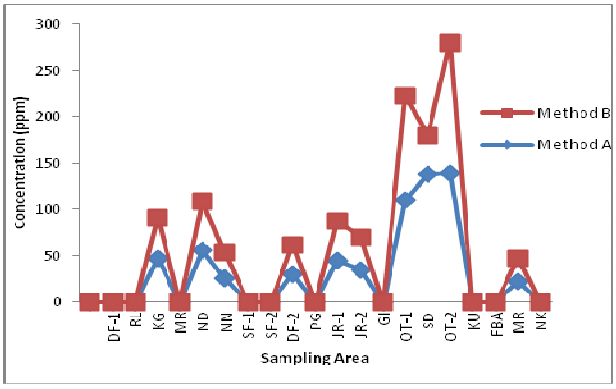

Fig. 6: Concentration of para red in chilli powder samples by method A & B

Table 2: Concentration of para red in chilli powder samples

S.No.	Sampling Area	Method A Concentration (ppm)	Method B Concentration (ppm)	TLC Results	
				Absent/Present	Rf
1.	DF-1 (Defence-1)	-	-	absent	-
2.	RL (Railway line)	-	-	absent	-
3.	KG (Korangi)	46.50	43.90	present	0.70
4.	MR (Malir)	-	-	absent	-
5.	ND (Nazimabad)	55.63	53.17	present	0.74
6.	NN (North Nazimabad)	25.21	28.04	present	0.74
7.	SF-1 (Shah Faisal-1)	-	-	absent	-
8.	SF-2 (Shah Faisal-2)	-	-	absent	-
9.	DF-2 (Defence-2)	30.63	30.48	present	0.69
10.	PG (Pahlwan Goat)	-	-	absent	-
11.	JR-1 (Gulstan-e-Johar-1)	45.21	42.68	present	0.68
12.	JR-2 (Gulstan-e-Johar-2)	34.17	35.36	present	0.73
13.	GI (Gulshan-e-Iqbal)	-	-	absent	-
14.	0T-1 (Orangi Town-1)	110.4	111.6	present	0.30
15.	SD (Saddar)	137.9	42.0	present	0.60
16.	0T-2 (Orangi Town-2)	139.5	140.0	present	0.60
17.	KU (Karachi University)	-	-	absent	-
18.	FBA (Federal B Area)	-	-	absent	
19.	MR (Malir)	22.3	24.2	present	0.70
20.	NK (North Karachi)	-	-	absent	-

Method A = Single Point External Standard Method, Method B = Multiple Point External Standard Method,
TLC = Thin Layer Chromatography, Rf = Retention Factor

Table 3: Concentration of para red in tomato sauces samples

S.No.	Sampling Area	Method A Concentration (ppm)	Method B Concentration (ppm)	TLC Results	
				Absent/Present	Rf
1.	NN (North Nazimabad)	-	-	absent	-
2.	DF-1 (Defence-1)	-	-	absent	-
3.	NK (North Karachi)	-	-	absent	-
4.	CL (Clifton)			absent	
5.	DF-2 (Defence-2)	-	-	absent	-
6.	SF (Shah Faisal)	-	-	absent	-
7.	SD-1 (Saddar-1)	99.95	100	present	0.61
8.	SD-2 (Saddar-2)	37.48	40.0	present	0.60
9.	SD-3 (Saddar-3)	-	-	absent	-
10.	GJ (Gulstan-e-Johar)	55.53	57.14	present	0.62
11.	MR (Malir)	-	-	absent	-
12.	GI (Gulshan-e-Iqbal)	3.30	3.28	present	0.70
13.	FBA (Federal B Area)	3.39	3.48	present	0.72
14.	SM (Sauabad Malir)	-	-	absent	-
15.	MC (Model Colony)	-	-	absent	-
16.	KT (Kausar Town)	-	-	absent	-
17.	OT (Orangi Town)	82.6	82.6	absent	0.72
18.	KG (Korangi)	-	-	absent	-
19.	KU (Karachi University)	-	-	absent	-
20.	RL (Railway line)	-	-	absent	-

Method A = Single Point External Standard Method, Method B = Multiple Point External Standard Method,

TLC = Thin Layer Chromatography, Rf = Retention Factor

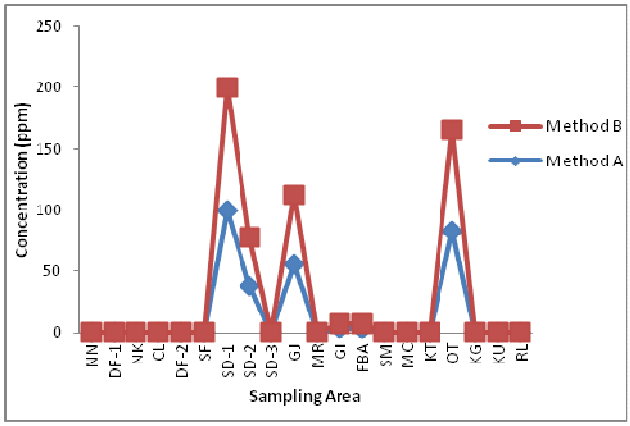

Fig. 7: Concentration of para red in tomato sauce samples by method A & B

It is evident from these results that the co-extracted component of the matrix may show false presence of para red dye in the detected range because *capsicum* (red chilli) carotenoids analogues also absorb in the same range as para red dye. To overcome this problem spiked standards were used. The Rf values of standard para red and sample extract were also compared on TLC plates. The similar values of retention factor of standard and sample on TLC plate confirmed the presence of para red in extracted sample. The method presented here is simplest, expeditious and highly economical. The method do not required sophisticated instrumentation which makes it superior to the other methods described earlier for initial studies of para red dye in chilli and tomato sauces.

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