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Abstract

Sudan I, II, III & IV are suspected genotoxic carcinogens and reports have shown the adulteration of chilli and chilli containing products with these azo dyes in recent past. In this study, a Simple Thin Layer Chromatographic method is described for the qualitative detection of Sudan-III & IV in Red chilli powder. The study includes extraction of sample, preparation of Thin Layer Chromatography (TLC) plates and Separation cum detection of dyes on TLC SODWHV 7KH G\HV ZHUH H[WUDFWHG ZLWK and relative R_f values. The relative R_f values of four samples 6DPSOH H[analysed, relative to the two standards of Sudan (III- S_2 and IV S_1) were less than one (P_1-0.13,0.25; P_2-0.19,0.35; P_3-0.30,0.35; P_4-0.15,0.26 and P_5 FRQ¿UPLQJ WKH DEVHQFH RI 6XGDQ ,,, DQG ,9 LQ layer chromatography in benzene, hexane and acetic acid solvent system. The chromatograms of sample, standard and spiked samples were evaluated in term of R HI¿FLHQF\ RI WKH PHWKRG ZDV FKHFNHG E\ LQWHUQDO VWDQGDUG PHWKRG LQ weight of standard dye. The relative R_f values of spiked sample was almost equal to one (b_1-0.93, b_2-0.80). Limit of detection (5 mg/kg) was calculated on the basis of visual evaluation of spots (i.e. spot visible or not at given concentration of standard dye) under normal light without any derivetization. The chilli samples analysed though varied in color intensity, but none of them was found to contain Sudan III or IV.

with Sudan IV (less o en with Sudan I) and originates from various African countries [2].

Sudan dyes are fat soluble azo dyes but solubility data of Sudan dyes is not abundant in literature. Sudan dyes are insoluble in water and soluble in various organic solvents (1.49 mol/dm3 in tri-chloromethane, 0.57 mol/dm^3 in dichloromethane, 0.30 mol/dm^3 in toluene, 0.17 mol/dm^3 in benzene, 0.04 mol/dm^3 in acetonitrile, 0.02 mol/dm^3 in ethanol and 0.017 mol/dm^3 in methanol. ere is no solubility information for Sudan II. Sudan III is 0.015 and Sudan IV is 0.09 mol/dm^3 soluble in ethanol.

e log-P value of Sudan I is 5.86 meaning that it is highly lipophilicity compound. No data is available for Sudan II-IV but given their molecular structures, their log-P values are expected to

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in the EU [4,5]. □ ey generate metabolites that are converted to several active mutagens and carcinogens in humans [6]. Sudan dyes are used in cosmetic products and animal testing has found that isomers of Sudan III cause allergic reaction [1].

Materials and Methods

Reagents and materials

Sudan III and Sudan IV were purchased from Merck. Acetonitrile, benzene, hexane, acetic acid and diethyl ether of HPLC grade was used. Silica gel TLC grade was used on plates of 10×10 cm. silica gel beaded HPLC grade was used for extraction of colors from the sample.

Standard solutions

Standard stock solutions were prepared by dissolving 5 mg/50ml of solvent (acetonitrile,) and stored at 10°C for one week. □ e working standard solutions were obtained from stock solutions by dilution (1:10) with the solvent.

Preparation of chilli samples

Four chilli samples were collected from the market including one pure control sample which was prepared by proper grinding of the whole pepper fruit to □ne powder. 5 grams of sample was dissolved in 25 ml of the solvent acetonitrile. 10 grams of silica gel (beaded) and 2 grams of anhydrous sodium sulphate was added. □ e mixture was properly stirred for 5 min on a magnetic stirrer. □ e coloring components from the silica gel were recovered by 2-3 volumes of diethyl ether (15-20 ml). □ e ethereal extract was evaporated in a porcelain dish on hot water bath. In order to separate the colors from the interfering matter, the dried extract was re-dissolved in 1ml of diethyl ether and applied as band on a preparatory TLC plate (thickness 1.0mm) the plate was developed in a chamber containing hexane, allowed to air dry and colors were scrapped into a conical □ask with addition of 10 ml of diethyl ether. □ e colored solution was □ltered and stored in dark bottles of 5 ml capacity.

Preparation of TLC plates

TLC plates of 10×10 cm were prepared with the help of spreader, having provisions for varying the thickness of silica gel layer from 0.25 mm to 2 mm. Slurry of silica gel was prepared in distilled water and applied as a thin layer 0.25mm on TLC plates, allowed to air dry and then activated in oven for 1 hr at 110°C.

Experiment

Samples and standards were applied as spots in triplicate using either capillary or micro-pipette on a prepared TLC plates (10×10). Distance from the le□ and right edge of the plate was 10 mm and from the lower edge was 8 mm. □ e distance between the spots was kept at 8 mm. 9 spots were applied on a single plate. □ e scheme of the spot application is shown in table 1. Development of the plate was performed in saturated TLC jar with di□erent solvent systems and the development time was 1½ hour. For visual evaluation the chromatograms were evaluated under normal light and the distances moved by solvent and the spots were measured by template scale (Figure 1).

Results and Discussions

Sudan IV and III were selected for the study because of their color, naturally occurring colorants, such as carotenoids which are extracted simultaneously. Hexane, ethanol, acetone, acetonitrile and diethyl ether were evaluated as extraction solvents and □nally samples were extracted in acetonitrile. Nevertheless when separated with selected chromatographic system (benzene; hexane; acetic acid 40:60:2), most chilli powder extracts showed considerable amount of colored matrix that could potentially interfere with the target compounds. In order to avert the interferences of the colored matrix, the samples (P_1, P_2, P_3 and P_4) were prepared on preparative TLC plates to separate di□erent compounds. □ e chromatograms of sample, standard and spiked sample are shown in □gure 1. □ e results were expressed in terms of R_f (retardation Factor) and Relative R_f values (Table 1). Mean of the R_f values were calculated from three triplicate spots of the given sample/standard. □ e standard S_1 and S_2 showed the grand mean R_f values 0.29 and 0.16 respectively. □ e relative R_f values of four samples analyzed were less than 1 (Table 1) con□rming absence of the suspected dyes S_1 and S_2 in all the samples.

□ e chromatogram of the sample (P_3, P_4) however showed two band separation. □ e top-most band (0f p3) was separated through preparative TLC method, eluted and further analyzed under UV-visible spectroscopy in wavelength scan mode. □ e wave length scan of the given spot however did not match with any of the standard (S_1 and S_2) (Figure 2).

□ e performance of sample extraction and TLC separation was cross checked by internal standard procedure, in which pure sample (P_1) was spiked by 5 mg/kg of standard dye (S_1 and S_2). □ e relative R_f values of both spiked samples b_1 and b_2 were almost equal to 1 (table 1). □ e limit of detection was based on visual evaluation of coloring spot. When standard dyes were spiked in control (pure chilli powder), the spots were visible up to 5 mg/kg.

Conclusion

• T e R_f values of coloring spots from all the samples selected did not matched with neither of standards (S1, S2).

• T e relative R_f values of all the sample color spot were less than one. Only one sample showed the relative R_f value of 0.5.

• T e R_f values of both spiked samples (b1, b2) were almost similar to their respective standards.

• T e relative R_f values of spiked samples were almost close to 1.

• T e limit of detection based on the visual evaluation of coloring spot was found to be 5mg/kg.

It is thus concluded that none of the four samples taken contained the suspected Sudan III or IV. □e variation of color intensity may be due to some other reason.

References

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