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Review

A review of analytical techniques for determination of Sudan I–IV dyes in food matrixes

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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. Due to the continuing illicit use of Sudan dyes as food colorants their determination in different food matrices – especially in different chilli and tomato sauces and related products – has during the recent years received increasing attention all over the world. This paper critically reviews the published determination methods of Sudan I–IV dyes. LC–UV–vis and LC–MS are the dominating methods for analysis of Sudan I–IV dyes. Sudan dyes are usually found in food at mg kg^{-1} levels at which it may be necessary to use a preconcentration step in order to attain the desired detection limits. Liquid–solid extraction is the dominating sample preparation procedure. In recent years it has been supplemented by ultrasonic-assisted extraction and pressurized liquid extraction. Various solid phase extraction types have been used for sample cleanup. The large majority of works use conventional C18 columns and conventional LC eluents. Traditionally the UV–vis absorbance detection has been the most frequently used. In the recent years MS detection is applied more and more often as it offers more reliable identification possibilities.

Contents

1. Introduction

Sudan dyes (Sudans I–IV, Sudan Red B, Sudan Red 7B, Sudan Red G, Sudan Orange G, Sudan Black, Dimethyl Yellow, Para Red, Toluidine Red and Orange II) are a family of compounds in the class of azo dyes that are used for different industrial and scientific applications (coloring of fuel, staining for microscopy, etc.). Because of their low cost and wide availability, Sudan dyes are also attractive as food colorants. However, due to their carcinogenicity they are banned for food usage in most countries, including in the EU [1]. Nevertheless, European Union Rapid Alert System for Food and Feed reports it being found in various foods [2]. This paper focuses on Sudan I, II, III and IV (Fig. 1).

Although concentration of Sudan I in 100–1000 mg kg^{-1} range is required to impact the color of chilli products [3], commonly reported levels of Sudan dyes are in the low mg/kg range [2]. Hence,

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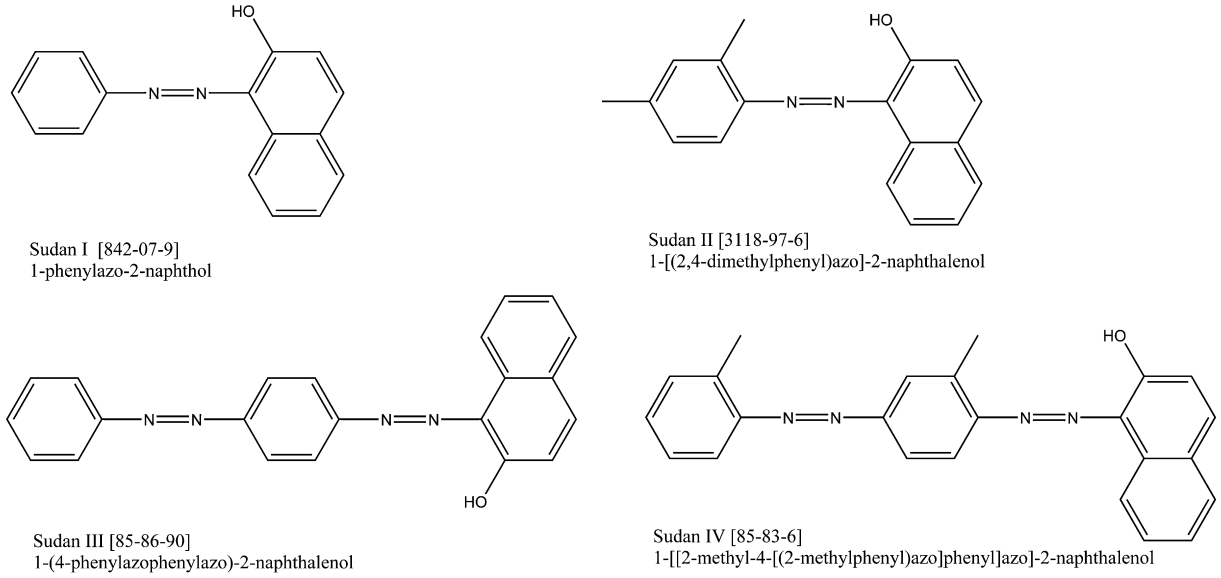

Fig. 1. Structures of Sudan dyes.

accurate determination of low levels of Sudan dyes in food is of huge importance.

There have been a large number of cases where Sudan dyes have been found in food, in particular, in the EU. This has forced the European Commission to adopt a decision on emergency measures against Sudan dyes in food and calling the member states to organize testing of food products on the market [4].

Due to the continuing use of Sudan dyes, their determination in different food matrices has received increasing attention all over the world in the recent years. A literature survey indicates that nearly 50 publications on Sudan dyes in food have been published between 2003 and 2009. The number of publications reached the peak in 2007 (Fig. 2). At the same time no review on determination of Sudan dyes has been published up to date.

Most of the determinations of Sudan dyes are carried out using liquid chromatography with different detection systems: UV–vis, electrochemical, and increasingly mass-spectrometric. A large variety of different sample preparation techniques have been used.

1.1. Chemical properties and toxicology of Sudan I–IV dyes

Sudan dyes are fat-soluble dyes but solubility data of Sudan dyes is not abundant in the literature. Solubility of Sudan I has been studied in Ref. [5]. It is insoluble in water and soluble in various organic solvents (1.49 mol/dm^3 in trichloromethane, 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). There is no solubility information for Sudan II. Sudan III is 0.15% and Sudan IV 0.09% soluble in ethanol [6]. According to Gurr, Sudan I–IV have very similar solubilities compared to each other [7].

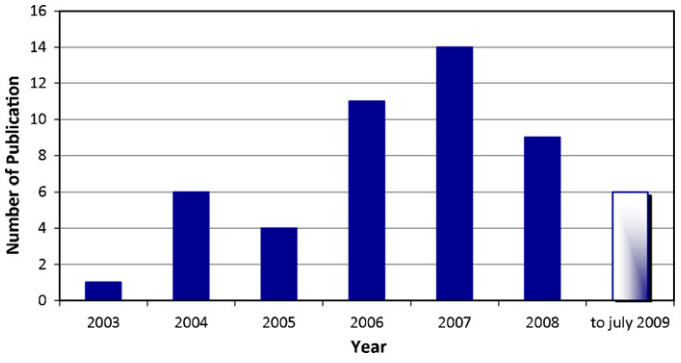

Fig. 2. Trends in number of publications of Sudan dyes analysis.

The log P value of Sudan I is 5.86 [5] meaning that it is a highly lipophilic compound. No data is available for Sudans II–IV, but given their molecular structures, their log P values are expected to be similar or somewhat higher.

UV spectra of the Sudan I–IV dyes differ. The absorbance maxima are at the following wavelengths: Sudan I 475 nm, Sudan II 489 nm, Sudan III 503 nm and Sudan IV 513–515 nm [7]. Sigma–Aldrich gives 2nd absorbances for Sudan II (604 nm) and Sudan IV (357 nm). For Sudan I it gives 1st absorbance 418 nm and 2nd absorbance 476 nm [8].

Because of their structure Sudan dyes display a number of isomeric equilibria. Rotational isomerism occurs at room temperature. Usually, this type of isomerism has no influence on the chemical analysis.

The azo-hydrazone prototropic tautomerism of Sudan I dye is illustrated in Fig. 3. Similar equilibria are possible in solutions of Sudan II–IV. In the case of Sudan I the hydrazone form predominates in formamide (73.8%), but in iso-octane 33.1% of hydrazone is present [9]. Prototropic tautomerism is known to be a fast process and normally rotational isomers are not separable by liquid chromatography.

Sudan dyes are analogues of azobenzene. By irradiation with UV light the E-form of azobenzene isomerizes to the Z-form (Fig. 4). In the absence of light the Z-form reverts thermally to the sterically less congested E-form. Possibility of similar E–Z isomerization processes in Sudan dyes and their analytical consequences have been briefly discussed [10].

Sudan dyes are weak acids. However, the azo group in the 1-position that acts as a hydrogen bond acceptor leads to formation of an intramolecular hydrogen bond with the phenolic OH. This additional stabilization of the neutral Sudan molecule is responsible for rather high pK_a value 11.65 [12] of Sudan I and II. The pK_a values of Sudan III and IV are not expected to be much different. One can thus conclude that throughout the pH range encountered in LC, Sudan dyes can be regarded as neutral molecules.

Sudan dyes are indirect carcinogens (classified as category 3 carcinogens by IARC) and are therefore banned from the use in food in the EU [1]. They generate metabolites that are converted to several active mutagens and carcinogens in humans [13], e.g. different