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Research Article

Method for simultaneous screening of Sudan I - IV by eight MRMs using hyphenated technique, liquid chromatography-electrospray-tandem mass spectrometry in ginger and curry powder matrixes

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Abstract

A method based on the reverse-phase (RP) liquid chromatography-tandem mass spectrometry interfaced with electrospray (LC-ESI-MS/MS) was developed for the detection and quantification of Sudan I-IV in the samples with ginger matrices. Sample extraction was done with acetonitrile acidified with formic acid. C18 column with an aqueous formic acid and acetonitrile acidified with formic acid mobile phase was used for separation under the gradient condition. Mass detection was done in positive polarity applying multiple reaction monitoring (MRM) of two fragmentation transitions per compound to get a high selectivity. The method was validated in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, sensitivity, accuracy, recovery and selectivity. A low level of 1µg/kg detection of the dye was achieved by using this technique. All the four dyes could be recovered from these matrices at 90-110% at a spike level of 10µg/kg.

Keywords: Sudan azo dyes; MRM, LC-ESI-MS/MS, food analysis, colourants, carcinogens, India

Introduction

Among more than 2000 synthetic organic colourants [1], azodyes are most widely used as artificial colourants. Sudan dyes are red dyes that are used for colouring solvents, oil, waxes, petrol, shoe polish, floor polish etc. Chemically they are nitrogen containing azo dyes which are fairly inexpensive and readily available and used for imparting uniform colour. The structures of Sudan I – IV are given in Figure 1.

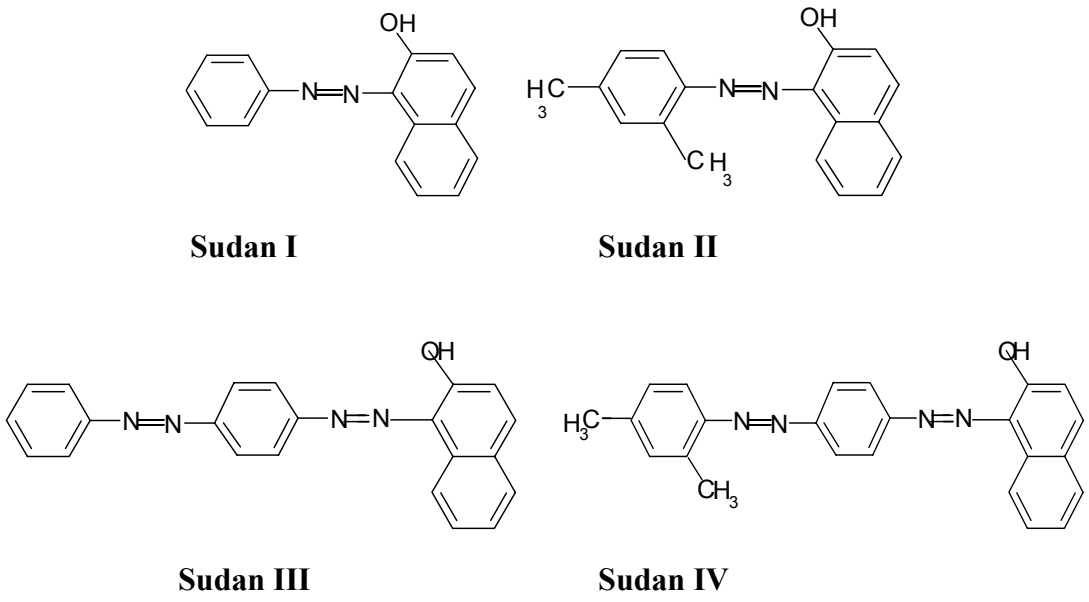

Figure 1. Structure of Sudan dyes.

Sudan dyes have been shown to cause cancer in laboratory animals and these findings could also be significant for human health. The International Agency for Research on Cancer (IARC, a part of WHO) has categorized some of the Sudan dyes as Group-3 carcinogens [2]. Moreover, most of the azo dyes are recognized to be carcinogens [3]. Hence they are legally banned for use the in food even at parts per billion (ppb) levels [4].

Illegal addition of these azo dyes is presumably done to improve the visual colour and appearance and also to retain the colour for a prolonged time. This dye gradually deteriorates into organic amines which are identified as genotoxic [2, 5].

The regulations of the European Commission (EC No. 178/2002) announced the general principles and requirements of food law [6, 7, 8, 9] in the market or use in food or feed which lead to serious risk to health. Based on these, a method for the analysis of Sudan dyes in chilli matrix was promulgated by the EU [10] as the adulteration was mainly detected in ground chillies.

To confirm the presence of the Sudan dyes, development of analytical methods is crucial. There are different methods for analysis of Sudan dyes. These include high performance liquid chromatography (HPLC) with a spectrophotometric detector [11] or flourescence detector [12]. The maximum absorbance (λ_{max}) of Sudan I and II is 476 nm and that of Sudan III and IV is 520 nm. Hence a detector which is capable of scanning both these wavelengths simultaneously is needed for the analysis of Sudan dyes. However, since the target compounds cause health hazards, there is a need for their determination at ppb levels which requires techniques that are more sensitive than conventional HPLC detection.

Liquid Chromatography coupled with Tandem Mass Spectrometry (LC MS/MS) is a sophisticated analytical tool for trace level analysis [13]. This technique relies on molecular level characteristics of the analyte and their behaviour towards the tandem mass spectrometer to produce highly specific and accurate results. The use of tandem techniques relies on the highly specific formation of daughter ions from the parent ions generated from the analytes, which makes this methodology highly reliable for trace work.

Several studies on analysis of Sudan dyes in other food matrices are reported [14, 15, 16]. However, no studies were reported for the analysis of these dyes in ginger matrices.

Materials and Methods

In the present study, LC MS/MS with Electro Spray Ionization (ESI) was used for the analysis of Sudan dyes in ginger and curry powder samples. Around 100 samples were taken for this study from different locations and with different compositions of curry powders as well as ginger. Eight MRM were used for the simultaneous quantification of Sudan I, Sudan II, Sudan III and Sudan IV.

The reference standards for Sudan I – IV were procured from Sigma Aldrich India (purity ≥ 98%). HPLC grade acetonitrile, chloroform, acetone and formic Acid were obtained from Merck India, and high purity, HPLC grade water was obtained using Barnstead Diamond® RO unit. Filter papers used were Whatman #1 Qualitative Circles (Cat 1001 150). Merck 0.45 disposable 0.45 micron syringe-filters were used for filtering the extract prior to instrumental analysis.

The API 2000 MS/MS system (AB Sciex) with Turbo Ion Spray (ESI), coupled to a Perkin Elmer quaternary gradient LC system with programmable autosampler, was used for the study. The column used was a Perkin Elmer RP-18 Shperisorb (220mm × 4.6mm × 5µm). Gradient elution was done with 0.2 % formic acid in water (A) and 0.2 % formic acid in acetonitrile (B). Flow rate was 1ml/min and injection volume was 20µl. 40°C was column oven temperature. MS/MS conditions are summarised in Table 1.

Table 1. MS/MS conditions.

MRM Transition	DP*	CEP*	CE*	CXP*
249 → 156	22	28	26	4
249 →128	22	28	35	4
277 →156	22	29	30	4
277 →120	22	29	28	4
353 →128	23	32	47	6
353 →156	23	32	36	3
381 →224	26	33	37	6
381 →106	26	33	47	3

* DP = Declustering Potential, FP = Focussing Potential (400V), EP = Entrance Potential (10V),
CEP = Collision Cell Entrance Potential, CE = Collision Entergy, CXP = Collision Cell Exit Potential
Curtain Gas = 30, Ion Source Voltage = 5500V, Temperature = 350°C, Nebuliser Gas = 75,
Heating Gas = 25

Stock solutions of 500 mg/kg of the dyes were prepared in eluent (B). In the case of Sudan III and IV, the dyes were first dissolved in a minimum quantity of HPLC chloroform before making up to required volume using acetonitirle. Serial dilutions were used to prepare working standards in the range 1.0 µg/kg to 1.0 mg/kg for preparing the linearity curve. Fortified samples were prepared to estimate recovery of the Sudan dyes from ginger matrix. Required amounts of the stock solutions of the dyes were added to ginger powder and curry powder samples to obtain spike levels of 10µg/kg and 100µg/kg.

About 10g of the samples were accurately weighed and transferred into 250 ml conical flasks. The samples were then extracted for 1 hour with 100ml acetonitrile using a platform shaker set at medium speed. The extracts were then filtered through Whatman #1 filter papers. The filtered extracts were further diluted 2.5 times and filtered through disposable 0.45 micron syringe-filters and 20µl of these diluted extracts were injected directly into the LC MS/MS system.

Results and Discussion

LC MS/MS (ESI, positive mode) of Sudan dyes were investigated in +ve polarity mode operating at a IS voltage of 5.5KV. The produced ion mass spectra of Sudan dyes showed a characteristic fragmentation pattern for all the analytes. In the case of Sudan I and Sudan II, a fragment corresponding to the loss of an OH^- was observed at m/z 232 and m/z 260, respectively [12] whereas in the case of Sudan III and Sudan IV such fragments were less intensive. The fragment ion at m/z 156 common to all Sudan dyes seems to be formed from the breaking of C-N bond to form $[C_{10}H_8N_2]^+$.

The LC MS/MS and HPLC-UV analysis of Sudan Dyes in chilli matrix has been reported elsewhere by Nagase, *et al*. [11] and Calbiani, *et al*. [14]. The chromatographic method used in this study showed good separation and mass compatibility. Under optimized LC conditions good separation was achieved within 15 minutes using a 25 cm column with good reproducibility. Figure 2 illustrates the LC MS/MS chromatographic separations of the four azo dyes with two MRM transitions each at concentration level of 1 µg/kg.

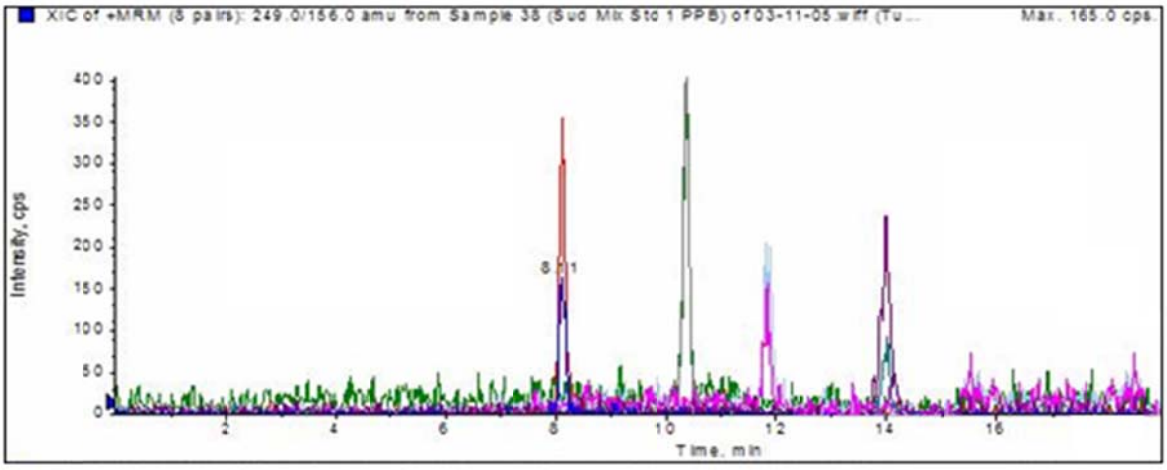

Figure 2. Chromatogram of standard Sudan dyes at 1µg/kg.

A series of matrix matched standards; spiked samples, matrix blanks and recovery samples were analyzed in order to evaluate the efficacy of the method. The reproducibility data is summarized in Table 2 and the linearity in Figure 3.

Table 2. Reproducibility measurements of Sudan dyes in the curry powder matrices.

No.	Compound	Spike Level (µg/kg)	Reproducibility (%CV) n=5
1	Sudan I	10	4.09
		100	9.93
		750	1.37
2	Sudan II	10	8.38
		100	7.46
		750	1.83
3	Sudan III	10	9.21
		100	6.78
		750	1.82
4	Sudan IV	10	9.39
		100	7.76
		750	3.12

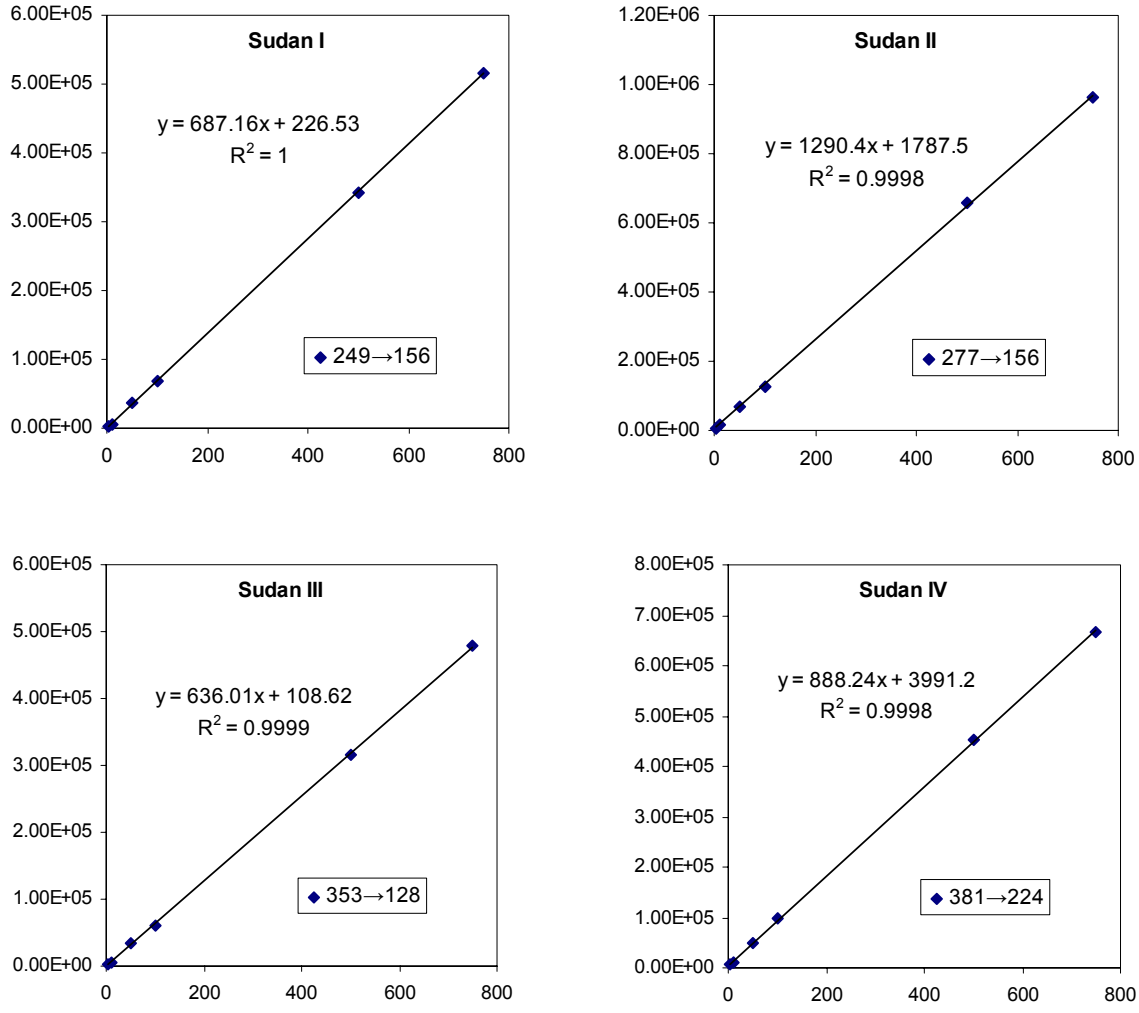

Figure 3. Linearity curves of Sudan dyes in spiked matrices.

Table 3. Recovery results from spike studies.

% Recovery	Spike Levels (µg/kg)							
	Sudan I		Sudan II		Sudan III		Sudan IV	
	10	100	10	100	10	100	10	100
Ginger Powder	97.7	80.8	101.0	82.1	92.4	90.4	102.0	102.0
Curry Powder	93.0	85.0	107.0	108.0	83.6	97.6	102.0	91.2

All the recoveries, from both ginger and curry powder matrices, were well within the stipulated control limits of 80 – 120%. The reproducibility result from Table 2 indicates good precision, as none of the %CV values have exceeded 10. This high level of precision, in tandem with the good recovery values in Table 3 testifies to the reliability of the analytical method.

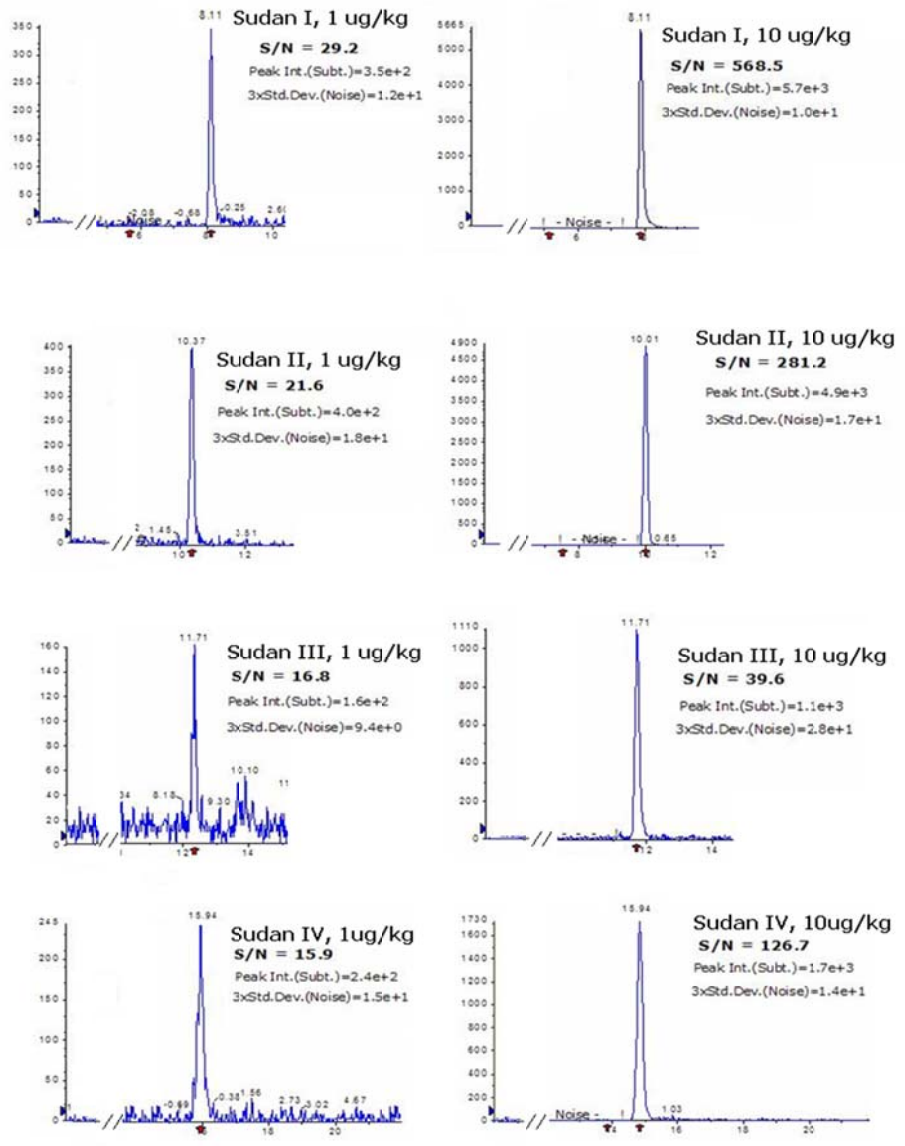

Figure 4. Determination of LOD/LOQ values for Sudan Dyes in curry powder.

Figure 4 shows the observed peak-to-peak S/N ratio for determination of limit of detection (LOD) and quantification (LOQ) of Sudan I to IV. As per norms, S/N ratio for LOD should be ≥3 and that for LOQ should be ≥10 [17]. As per the present study, the lowest matrix matched calibration standard (1 µg/kg) gave S/N ratio well above the requirement of even the LOQ.

Conclusion

The results of the study indicated that the method under consideration was well suited for the analysis of trace level Sudan contamination in ginger and curry powder. These two matrices are known to be particularly difficult ones for trace work; hence it is presumed that the method which works well for these two matrices can also be extended to other spice matrices as well without much difficulty.

Acknowledgement

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