

# Analysis of Illegal Dyes in Food Matrices using Automated Online Sample Preparation with Liquid Chromatography-Mass Spectrometry

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## Overview

**Purpose:** To develop a rapid and sensitive automated online sample preparation LC-MS/MS method to detect and quantify multiple Sudan dyes in a variety of matrices and also to shorten assay time and increase throughput.

**Methods:** Automated online sample preparation using Thermo Scientific TurboFlow technology coupled with the Thermo Scientific Exactive benchtop Orbitrap mass spectrometer.

**Results:** A TurboFlow™ online multi-residue screening method for complicated food matrices utilizing full scan accurate mass detection was developed.

## Introduction

Sudan dyes are red dyes used for coloring solvents, oils, waxes, petrol, or as additives in shoe and floor polish. In addition, they have been found in a number of food products such as chili or chili-containing products. Sudan dyes are banned as food additives in the USA<sup>1</sup>, the EU<sup>2,3</sup>, and many other countries, due to their links to cancer and other negative health effects.

Liquid chromatography-ultraviolet-visible (LC–UV–vis) and liquid chromatography-mass spectrometry (LC–MS) are currently the most popular methods for analysis of Sudan dyes. Traditional sample preparation methods, especially solid phase extraction (SPE), have been widely used in the determination of Sudan dyes. However, these procedures can be labor-intensive, time-consuming and costly, resulting in low sample throughput when performed manually. Lower recoveries have been also noticed associated with SPE cleanup.<sup>4</sup> There is consensus that one of the major scientific challenges in Sudan dyes analysis is to achieve high sensitivity and selectivity while keeping minimal sample clean up.<sup>5</sup>

In this study we describe an easy, comprehensive LC method using a Thermo Scientific Transcend TLX-1 system powered by TurboFlow technology to analyze five illegal dye residues in a variety of sauces.

## Methods

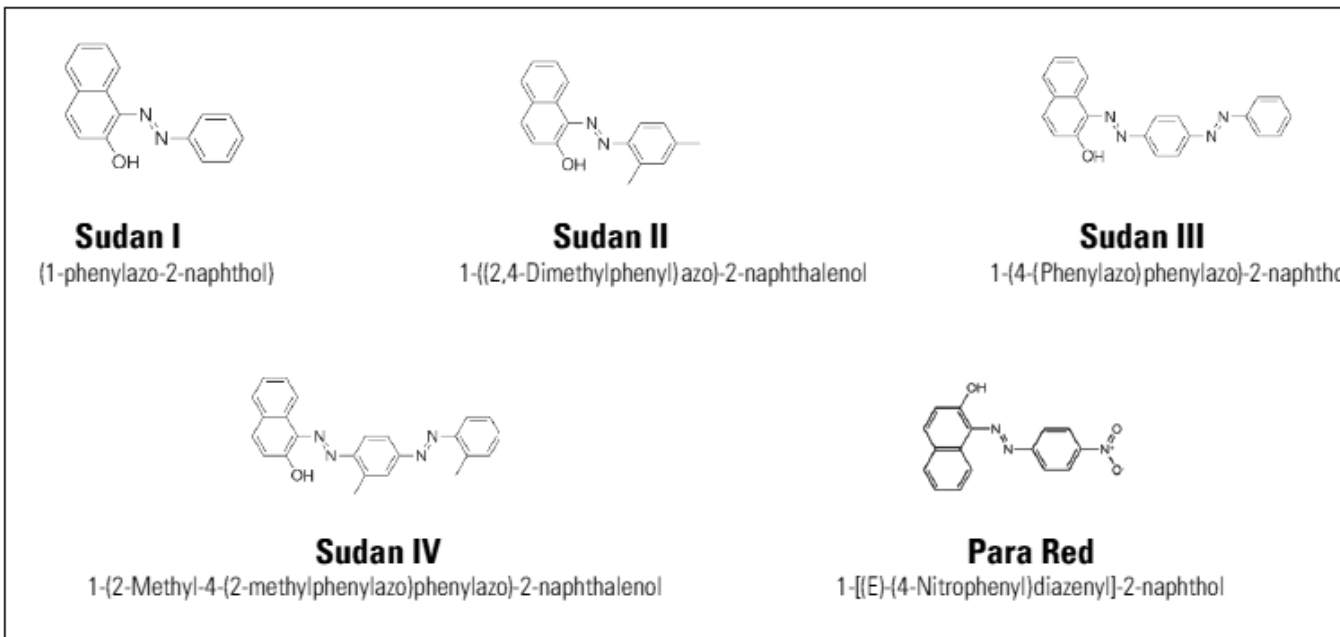
### The matrix standard curve

Five analytes, Sudan I, Sudan II, Sudan III, Sudan IV and Para Red (Figure 1) were obtained from Sigma Aldrich (St. Louis, MO). A total of four different food products purchased from local grocery stores were used in this study: Chili Sauce I; Chili Sauce II; Hot Sauce I; Hot Sauce II.

Three grams of each homogenized matrix were weighed into a 50 mL centrifuge tube, followed by the addition of 30 mL acetonitrile (ACN). The tube was vortexed for 10 minutes and then sonicated for another 60 minutes. The resulting solutions were centrifuged at 10,000 RPM for 15 minutes. The supernatant was then filtered through a 0.45 mm syringe filter. No additional clean up of the sample solution was performed. Each milliliter of supernatant corresponds to 0.1 g semi-solid food matrix as the unit of conversion.

A calibrant stock solution was prepared at a final concentration of 1 mg/mL of each analyte in ACN. A range of calibration solutions from 0.5 to 100 ng/mL (equals to 5 ng to 1000 ng/g) were made by serial dilutions using individual produced supernatants.

FIGURE 1. Chemical structure of test compounds



### LC/MS Methods

#### Thermo Scientific TurboFlow Method Parameters

Column: TurboFlow XL C8 column 0.5 x 50 mm  
Injection Volume: 25 µL  
Solvent A: 0.1% formic acid in Water  
Solvent B: 0.1% formic acid in ACN  
Solvent C: 1:1  
ACN: isopropanol

#### HPLC Method Parameters

Analytical Column: Thermo Scientific Accucore Phenyl Hexyl 3X50 mm 2.6 µm  
Solvent A: 0.1% formic acid in water  
Solvent B: 0.1% formic acid in ACN

The interference molecules from the matrix were unretained and moved to waste during the loading step of TurboFlow column, while the analyte of interest was retained on the extraction column. This was followed by organic elution to the analytical column and gradient elution to the MS.

### Mass Spectrometer Parameters

MS: Thermo Scientific Exactive benchtop Orbitrap™ MS  
MS Ionization Source: Heated Electrospray Ionization (H-ESI)  
Scan Range: 240.0 to 390.0 m/z  
Resolution: 50,000  
Spray Voltage: 4 kV  
Sheath Gas (N<sub>2</sub>): 70 arbitrary units  
Aux Gas (N<sub>2</sub>): 40 arbitrary units  
Heater Temperature: 400 °C  
Capillary Temperature: 350 °C  
Capillary Voltage: 27.5  
Tube lens Voltage: 95 V  
Skimmer Voltage: 22 V  
Polarity: Positive

### Data Analysis

The system was controlled by Thermo Scientific Aria OS software. Data acquisition was performed using Thermo Scientific Xcalibur software. The resulting data were processed with Thermo Scientific LCQUAN quantitative software. The accurate masses of analytes are listed in Table 1.

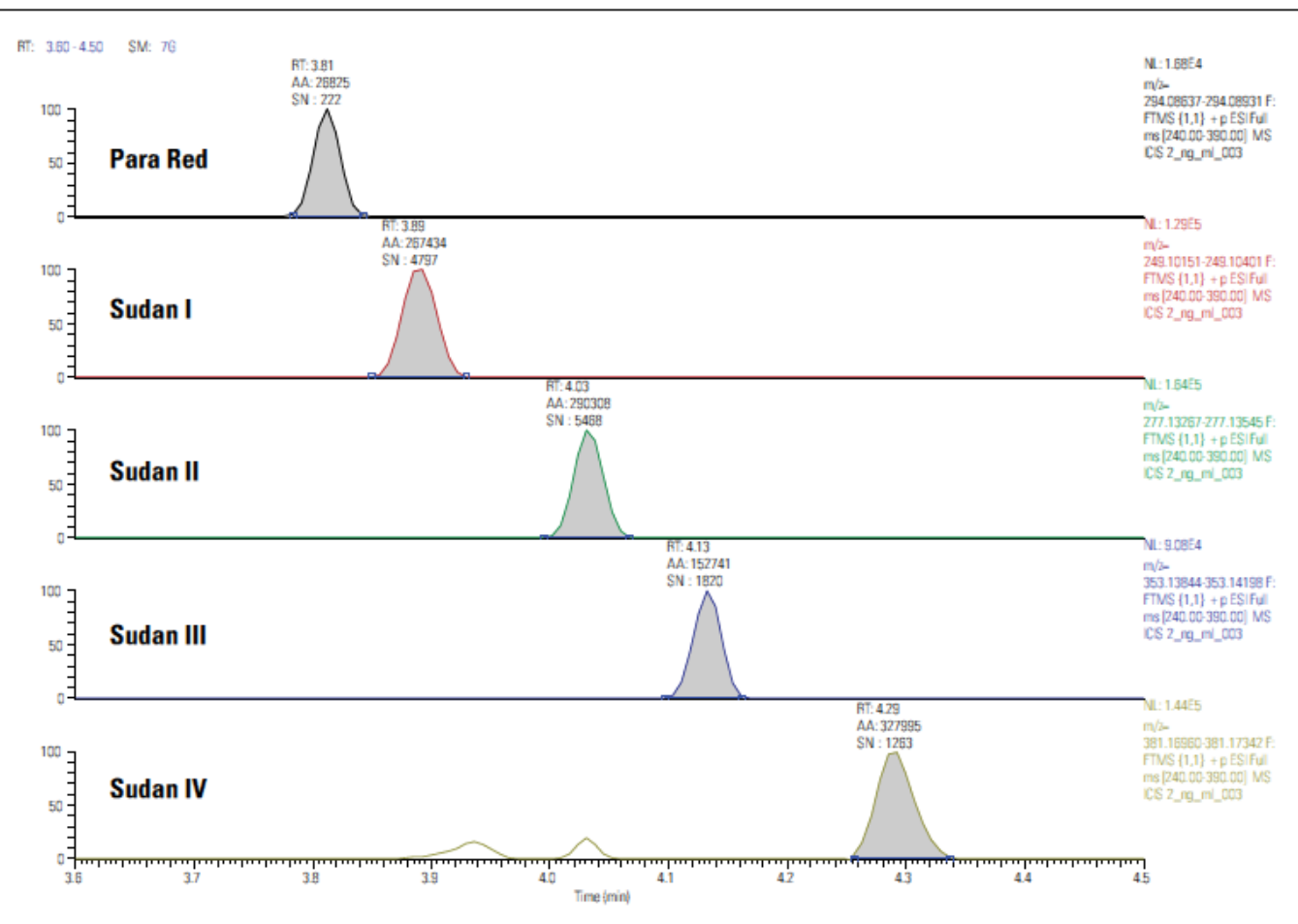
TABLE 1. Molecular formula, theoretical mass of test compounds

Compound	Formula	Exact Mass (m/z)	[M+H] <sup>+</sup> (m/z)
Para red	C <sub>18</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub>	293.080041	294.087841
Sudan I	C <sub>18</sub> H <sub>12</sub> N <sub>2</sub> O	248.094963	249.102763
Sudan II	C <sub>18</sub> H <sub>16</sub> N <sub>2</sub> O	276.126263	277.134063
Sudan III	C <sub>22</sub> H <sub>16</sub> N <sub>4</sub> O	352.132411	353.140211
Sudan IV	C <sub>28</sub> H <sub>20</sub> N <sub>4</sub> O	380.163711	381.171511

## Results and Discussion

Figure 2 shows the representative chromatograms of 5 analytes at 20 ng/g (2 ng/mL) in hot sauce II extract. For the concentration range studied (5-1000 ng/g), limits of quantitation (LOQs) were estimated from triplicate injections (coefficient of variation <15%) of standard solutions at concentration levels. Both the area precision and mean accuracy were below 20% at LOQ. As shown in Table 2, LOQs ranged from 5-20 ng/g for all analytes except para red in 4 studied sauces. A lower LOQ could be achieved by increasing sample injection volume because TurboFlow columns can handle larger injections (up to a few hundred microliters) while regular high performance LC (HPLC) or Ultra HPLC (UHPLC) columns cannot. Good linearity was observed over the entire tested range. The correlation coefficients obtained using weighted (1/x) linear regression analysis of standard curves were greater than 0.99 for all analytes.

FIGURE 2. Representative chromatogram (20 ng/g in Hot Sauce II)



To further assess the reproducibility of the present methodology, a relative standard deviation (%RSD) test was performed on all matrices fortified with analytes at 100 ng/g. Table 2 indicates that the RSDs of six replicate injections were less than 10% for the majority of analytes. These results show the feasibility of the current approach for dyes determination in food matrices.

A recovery study was performed on the four matrices fortified with analytes at 100 ng/g. The recovery was assessed by comparing the detector response of a post-extracted spiked sample with that determined from a spiked neat standard sample at the same concentration. As shown in Figure 3, recoveries were 80-120% for most analytes in all matrices except chili sauce II extract, which indicates no significant matrix effects for the majority of analytes. These matrix-matched calibration curves can be used to overcome matrix effects and calculate concentrations of these illegal dyes in routine lab work.

TABLE 2. Quantitation limit, linearity and relative standard deviation (%RSD) of analytes in four tested matrices

### Chili Sauce I

Compound	LOQ (ng/g)	R <sup>2</sup>	%RSD (n=6 at 100 ng/g)
Para Red	20	0.9955	9.64
Sudan I	10	0.9960	3.64
Sudan II	10	0.9936	6.24
Sudan III	10	0.9937	7.45
Sudan IV	5	0.9911	4.55

### Chili Sauce II

Compound	LOQ (ng/g)	R <sup>2</sup>	%RSD (n=6 at 100 ng/g)
Para Red	50	0.9900	4.50
Sudan I	10	0.9906	6.26
Sudan II	10	0.9920	10.82
Sudan III	10	0.9942	10.29
Sudan IV			

The data for Sudan IV was not quantifiable.

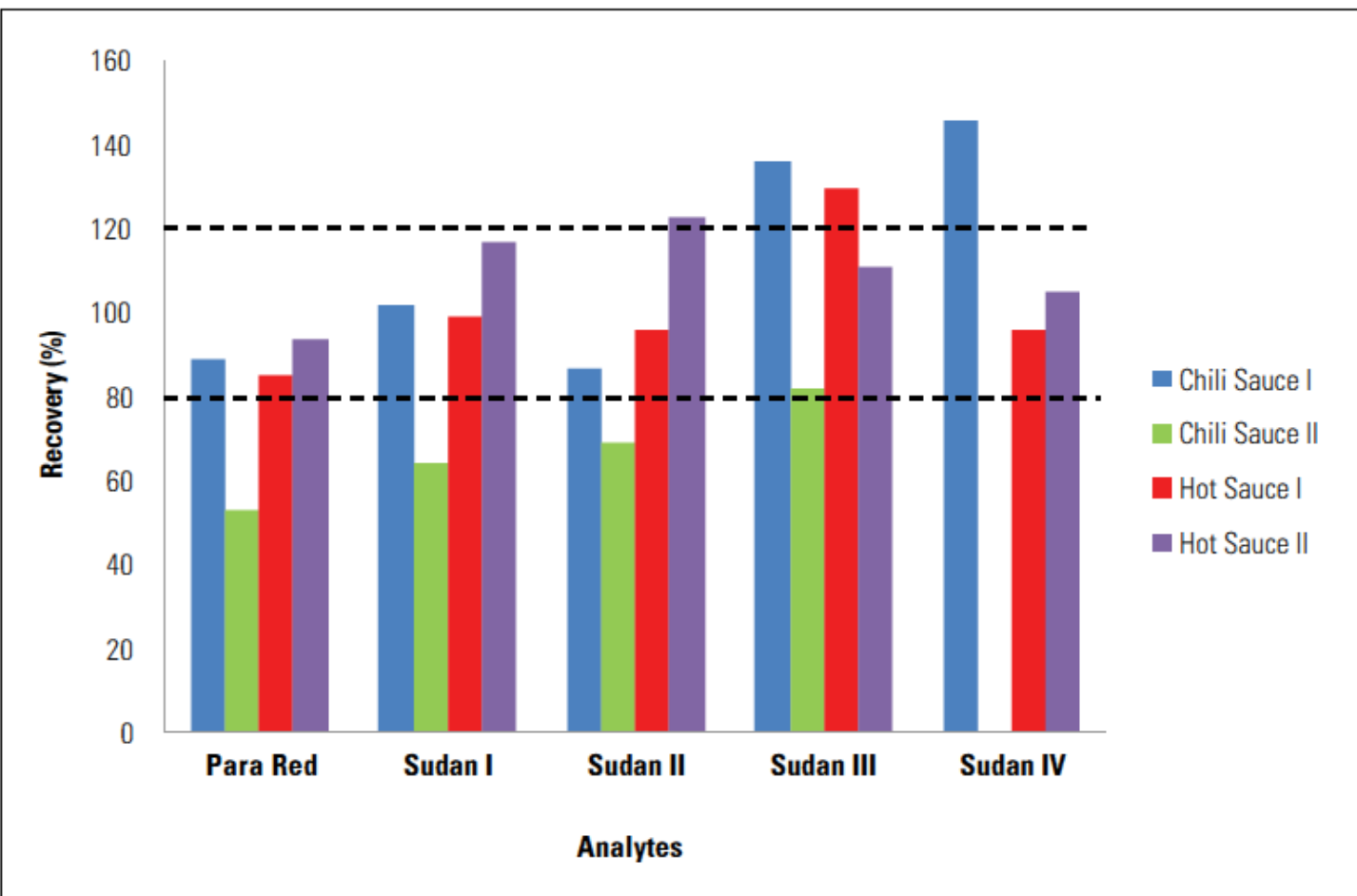
### Hot Sauce I

Compound	LOQ (ng/g)	R <sup>2</sup>	%RSD (n=6 at 100 ng/g)
Para Red	20	0.9952	4.92
Sudan I	5	0.9980	2.88
Sudan II	10	0.9969	5.11
Sudan III	10	0.9959	2.21
Sudan IV	20	0.9980	4.35

### Hot Sauce II

Compound	LOQ (ng/g)	R <sup>2</sup>	%RSD (n=6 at 100 ng/g)
Para Red	20	0.9973	7.62
Sudan I	5	0.9981	2.66
Sudan II	5	0.9976	5.36
Sudan III	5	0.9970	6.23
Sudan IV	5	0.9974	2.95

FIGURE 3. Recoveries of 5 analytes fortified in all tested matrices at 100 ng/g



## Conclusion

The current method has been tested with four different sauces. Linearity, specificity, recovery and repeatability of the method have been established. Sample preparation time of this strategy was minimal. Not including sonication and centrifugation times, the sample preparation only took 15 minutes. Additionally, since all analytes were eluted within less than one minute of a total six-minute LC run, multiplexing with a Transcend TLX-4 system would further reduce total LC-MS/MS run time four-fold and enable screening of more than 30 samples per hour. Future work could involve screening a larger range of illegal dyes, thus combining a screening method with accurate quantification.

## References

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