Application Note: 560

Analysis of Illegal Dyes in Food Matrices using Automated Online Sample Preparation with LC/MS

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Key Words

- Transcend TLX-1
- TurboFlow Technology
- Exactive
- Accucore HPLC Columns

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[1], the EU[2,3] and many other countries due to 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.[4] Traditional sample preparation methods, especially solid phase extraction (SPE), have also 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 also been noticed associated with SPE cleanup.[4] There is consensus that one of the major scientific challenges in the analysis of Sudan dyes is to achieve high sensitivity and selectivity while minimizing sample clean up.[5]

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

Goal

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

Experimental

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.

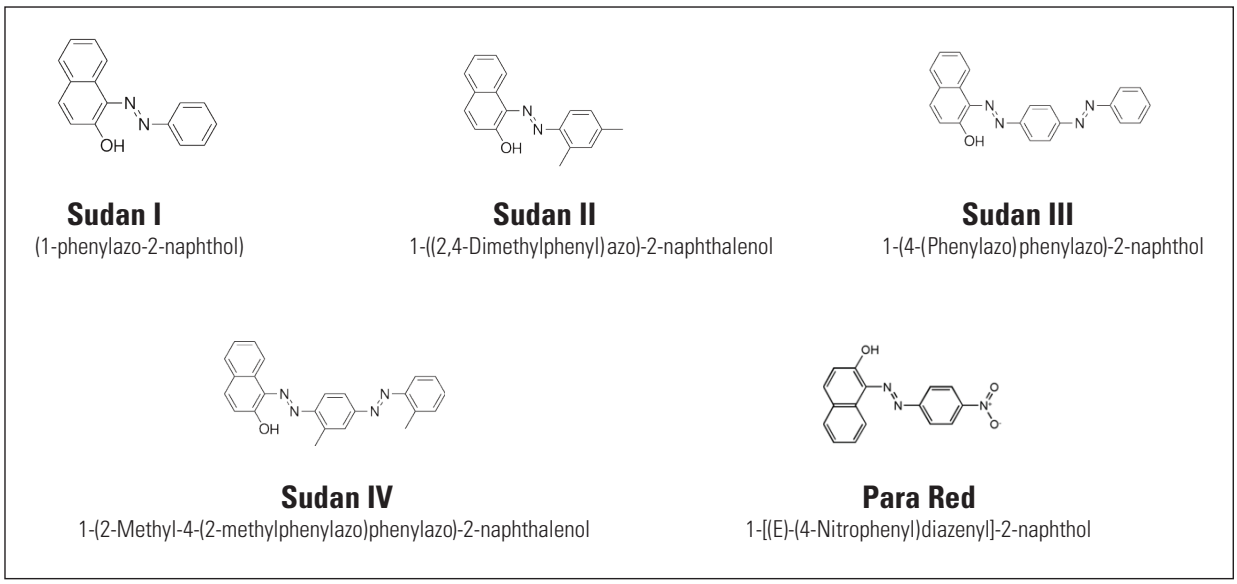

Figure 1. Chemical structure of test compounds

Three grams of each homogenized matrix were weighed into a 50-mL centrifuge tube, followed by the addition of 30 mL of acetonitrile (ACN). The tube was vortexed for 10 minutes and then sonicated for another 60 minutes. The resulting solution was 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 (equal to 5 to 1000 ng/g) was made by serial dilutions using individually produced supernatants.

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 50 x 3 mm, 2.6 µm particle size
Solvent A:	0.1% formic acid in water
Solvent B:	0.1% formic acid in ACN

Thermo Scientific Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 µm diameter of the particles results in much lower backpressures than typically seen with sub-2 µm materials.

Mass Spectrometer Parameters

MS:	Thermo Scientific Exactive high performance benchtop Orbitrap™ MS
MS Ionization Source:	Heated Electrospray Ionization (H-ESI)
Ionization Mode:	Positive
Scan Range:	*m/z* 240.0 to 390.0
Resolution:	50,000
Spray Voltage:	4 KV
Sheath Gas Pressure (N_2):	70 arbitrary units
Auxiliary Gas Pressure (N_2):	40 arbitrary units
Heater Temperature:	400 °C
Capillary Temperature:	350 °C
Capillary Voltage:	27.5 V
Tube Lens Voltage:	95 V
Skimmer Voltage:	22 V

The interference molecules from the matrix were unretained and moved to waste during the loading step of the TurboFlow column, while the analyte of interest was retained on the extraction column. This was followed by organic elution of the analytes to the analytical column and gradient elution to the MS. The system was controlled by Thermo Scientific Aria OS. Data acquisition was performed using Thermo Scientific Xcalibur software. The resulting data were processed with Thermo Scientific LCQUAN quantitative software. The accurate masses of the analytes are listed in Table 1.

Table 1. Testing compounds

	Formula	Exact Mass	$[M+H]^+$
Para Red	$C_{16}H_{11}N_3O_3$	293.080041	294.087841
Sudan I	$C_{16}H_{12}N_2O$	248.094963	249.102763
Sudan II	$C_{18}H_{16}N_2O$	276.126263	277.134063
Sudan III	$C_{22}H_{16}N_4O$	352.132411	353.140211
Sudan IV	$C_{24}H_{20}N_4O$	380.163711	381.171511

Results and Discussion

Figure 2 shows the representative chromatograms of the 5 analytes at 20 ng/g (2 ng/mL) in Hot Sauce II extract. For the concentration range studied (5-1000 ng/g), all limits of quantitation (LOQs) were estimated from triplicate injections (coefficient of variation < 15%) of standard solutions. The area precision and mean accuracy were below 20% at LOQ. As shown in Table 2, the LOQs ranged from 5-20 ng/g for all analytes except Para Red in four of the sauces studied. A lower LOQ could possibly 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 of each analyte. The correlation coefficients obtained using weighted (1/x) linear regression analysis of standard curves were greater than 0.99 for all analytes.

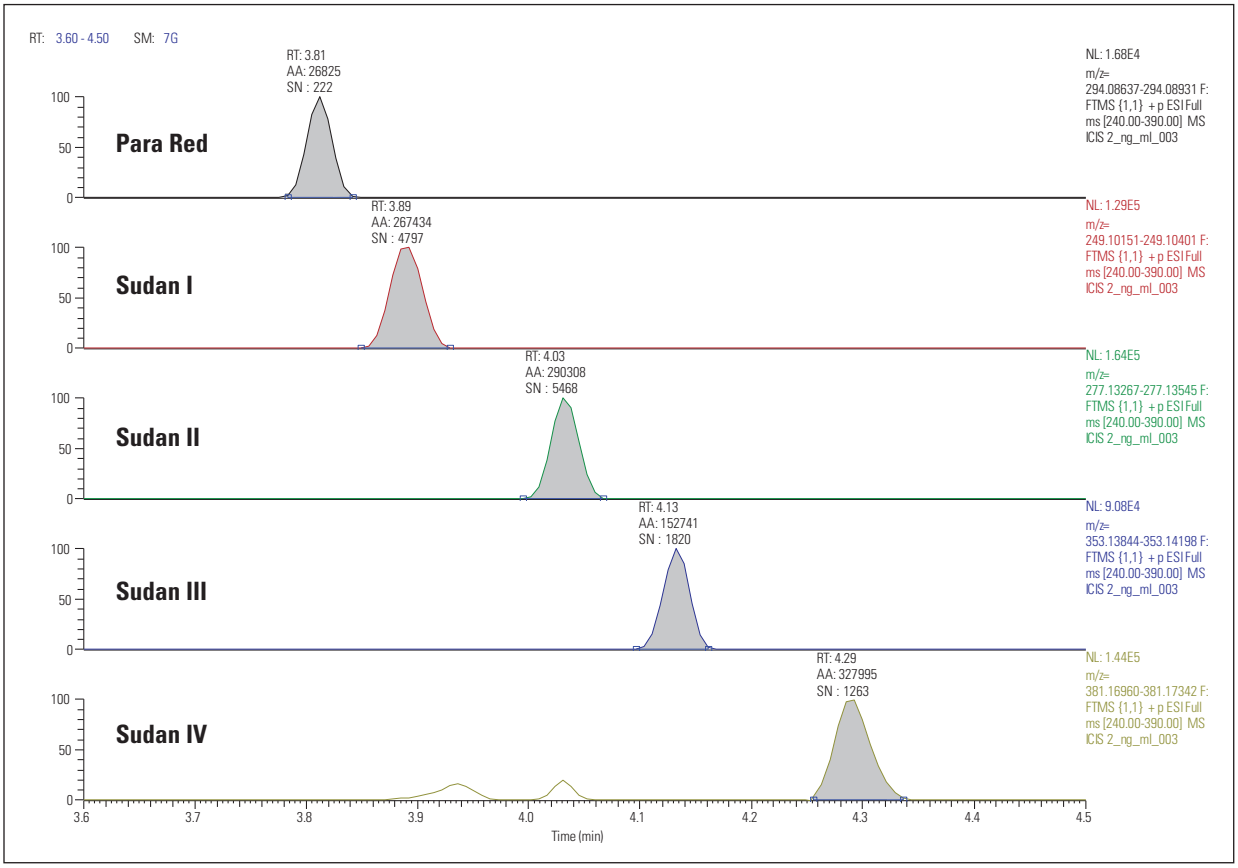

Figure 2. Representative chromatogram (20 ng/g in Hot Sauce II)

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 Sudan dyes determination in food matrices.

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

Chili Sauce I

	LOQ (ng/g)	R^2	%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

	LOQ (ng/g)	R^2	%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

	LOQ (ng/g)	R^2	%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

	LOQ (ng/g)	R^2	%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

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.

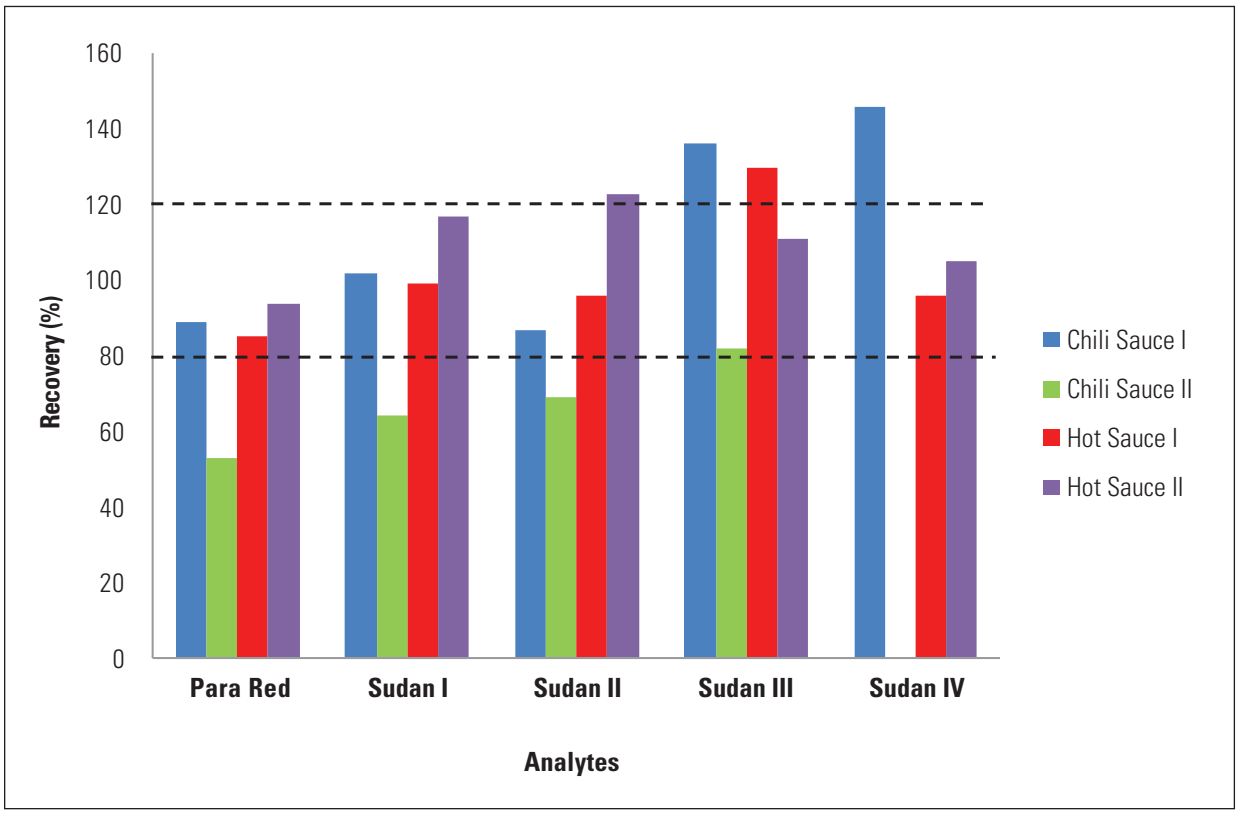

Figure 3. Recoveries of 5 analytes fortified in all tested matrices at 100 ng/g

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

1 U.S. Title 21 of the Code of Federal Regulation Part 73, Subpart A.

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