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 method to detect and quantify Sudan dyes and other illegal dyes in food matrices.

Methods: Automated online sample preparation using Thermo Scientific TurboFlow technology coupled with the Thermo Scientific Exactive benchtop orbitrap MS for detection of Sudan dyes.

LC/MS Methods
Detection strategy: TurboFlow™ online multi-residue screening method for complicated food matrices was developed.

Column: TurboFlow XL C8 column 0.5 x 50 mm

Solvent A: 0.1% formic acid in water
Solvent B: 0.1% formic acid in ACN
Solvent C: 1:1 ACN:isopropanol

Flow rate: 0.3 mL/min
Injection volume: 10 μL
Injection type: TurboDirect™

Column heater temperature: 400 °C
Spray heater temperature: 250 °C
Capillary heater temperature: 350 °C
Capillary voltage: 27.5 V
Spray voltage: 4 KV
Sheath gas: Heated Electrospray Ionization (H-ESI)
Sheath gas (N2): 70 arbitrary units
Tube lens voltage: 95 V
Resolution: 50,000
Scan range: 240.0 to 390.0 m/z
Polarity: Positive

Data Analysis:
The system was monitored by Thermo Scientific Xcalibur software.

MS Ionization Source: Heated Electrospray Ionization (H-ESI)
MS: Thermo Scientific Exactive benchtop Orbitrap™ MS
MS Ionization: Positive
Sheath Gas (N2): 70 arbitrary units
Sheath Gas Flow: 10 L/min
Spray Heater Temp: 250 °C
Orifice Heater Temp: 250 °C
Spray Voltage: 4 KV
Spray Lens Voltage: 95 V
Resolution: 50,000
Scan Range: 240.0 to 390.0 m/z
Polarity: Positive

In this study we describe an easy, comprehensive LC method using a Thermo Scientific TurboFlow™ system powered by TurboFlow technology to analyze illegal dyes in food matrices, including a variety of spices.

Methods

The matrix standard curve:
Five standards (Sudan I, II, III, IV, and Sudan Red) were prepared by dissolving weighed amounts of the dyes in appropriate solvents. Each standard solution was weighed into a 50 mL centrifuge tube and diluted to give 100 ng/g in the final solution. The tube was vortexed for 10 minutes and then sonicated for another 60 minutes. The resulting solutions were analyzed by LC/MS. The results were expressed as the percentage of the area of each compound relative to the internal standard. Each replicate was run in triplicate.

A calibration stock solution was prepared at a concentration of 1 mg/mL in methanol. A range of calibration solutions from 0 to 1000 ng/g in 100 ng/g steps were added to 10 mL of serial dilution using individual purchased analytes.

Data Analysis:
All standard solutions were injected in triplicate. Results were expressed as the percentage of the area of each compound relative to the internal standard. The data was analyzed using Thermo Scientific Xcalibur software. The results were expressed as the percentage of the area of each compound relative to the internal standard.

Results and Discussion

Figure 2 shows the representative chromatograms of a sample at 20 ng/g (2 ng/mL) for the Sudan dyes. 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 15% for the majority of analytes. These results show the feasibility of the current method for analysis of Sudan dyes in food matrices.

Conclusion
The current method has been tested with four different sauces. Linearity, specificity, sensitivity, and accuracy of the method were established. The analysis time of the strategy was minimal. Not including sonication and centrifugation times, the sample cleanup takes less than 15 minutes. Additionally, all analytes were easily resolved from matrix components. The lower detection limits for both HPLC and UHPLC systems encourage use of these products in any manners that might infringe the intellectual property rights of others.

References
1. U.S. Title 21 of the Code of Federal Regulation Part 72, Subpart A