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ORIGINAL ARTICLE

Fast and simple method for the detection and quantification of 15 synthetic dyes in sauce, cotton candy, and pickle by liquid chromatography/tandem mass spectrometry

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Abstract A simple and sensitive liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the simultaneous determination of 15 illegal dyes (Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Red G, Sudan Orange G, Sudan Red 7B, Para Red, Dimethyl Yellow, Rahodamine B, Sudan Black B, Sudan Red B, Auramine O, Toluidine Red and Orange II) was developed and validated in sauce, cotton candy, and pickle. The samples were extracted with acetonitrile without the use of solid-phase extraction cartridges. Chromatographic separation was achieved on a Zorbax Eclipse Plus C18 column with a flow rate of 500 µL/min at 45 °C, using a gradient elution with A (10 mM ammonium formate in water with 0.1% formic acid) and B (10 mM ammonium formate in acetonitrile (ACN) with 0.1% formic acid) as the mobile phase. The detection was performed on a AB Sciex 6500 Qtrap mass analyzer under multiple reaction monitoring mode. Limit of detection, quantification, linearity, and precision were determined during the validation process. Recoveries ranged from 82% to 119% for all synthetic dyes, in exception to Orange II in cotton candy and pickle, where signal was suppressed due to high matrix interference and poor ionization. This method offers a simple and rapid approach to detect and quantify prohibited dyes in foodstuff that can be utilized in food contaminant laboratories.

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1. Introduction

Synthetic dyes are chemical compounds used in food and pharmaceutical industries for appearance enhancement purposes due to their effectiveness, stability and low cost (Ma et al., 2006; Vachirapatama et al., 2008). Azo dyes, such as Sudan I – IV, are known class 3 carcinogens (IARC, 1975) and their

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use as a food coloring agent is mostly illegally worldwide (Zhu et al., 2014). However, these colorants are still commonly reported in many imported food products (Abdelmigid, 2009). Following the detection of several Sudan dyes in chilli powder, spice mixtures and sauces imported from India, Pakistan, Thailand, Lebanon and Turkey, the European Union (EU) adopted emergency measures regarding the application of synthetic dyes in foodstuff (Directive, 2003/460/EC). Regulations require that all food products exported to the EU be certified as fraudulent dyes free.

Several methods have been developed to detect synthetic dyes in multiple food matrices. Among the reported methods, high performance liquid chromatography (HPLC) coupled with ultraviolet (UV) or diode array detection (DAD) is the most common (Zheng et al., 2007; He et al., 2007). In addition, thin layer chromatography (TLC) and spectrophotometry are also popular approaches considering their low cost (Oka et al., 1994; Zalacain et al., 2005). However, these methods usually introduce complications due to their low sensitivity and high interferences. Liquid chromatography/tandem mass spectrometry (LC-MS/MS) and gas chromatography/mass spectrometry (GC–MS) methods have shown to be reliable, but the solid- phase extraction (SPE) involved in the sample preparation is time-consuming (Calbiani et al., 2004; Wanget al., 2008).

To the authors knowledge there is only one published method to detect synthetic dyes by LC-MS/MS that does not require SPE (Tsai et al., 2015). Tsai et al. (2015) method uses 10 g of sample and the analysis time exceeds 20 min, which are considered disadvantages when accommodating large surveillance programs. Also, the method did not include auramine O, Sudan black, Rhodamine B, Toluidine Red and Orange II, which are prohibited additives of great significance to food safety.

Saudi Arabia imports large quantities of food from south east Asia; therefore, it was crucial to develop a simple and practical method to ensure consumers' safety against fraudulent colorants. In this study, a rapid analytical method for the detection and quantifying of 15 illegal dyes in sauce, cotton candy, and Pickle by LC MS/MS was developed. The proposed method offers a single-step extraction and a 5 min analysis run time.

2. Material and methods

2.1. Reagents and chemicals

HPLC grade acetonitrile (ACN), HPLC grade water, Dichloromethane (DCM), Ethanol, ammonium formate (≥99.0%), and formic acid (≥98%) were purchased from Merck (Darmstadt, Germany). Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Red G, Sudan Orange G, Sudan Red 7B, Para Red, Dimethyl Yellow, Rahodamine B and Orange II were obtained from the Institute of Leather Industry (Łódź, Poland). Sudan Black B and Sudan Red B were purchased from Dr. Ehrenstorfer GmbH Company (Augsburg, Germany), and Auramine O and Toluidine Red were obtained from Sigma Aldrich (St. Louis, MO, USA) (Fig. 1).

2.2. Instrument

The LC-MS/MS system consisted of an ACQUITY UPLC I-Class System (Waters Technologies USA) equipped with a Binary Solvent Manager, Sample Manager - Flow-Through-Needle and a column heater with active pre-heating coupled to a 6500 Qtrap mass analyzer (AB Sciex, Canada). Nitrogen was used as curtain gas, nebulizer gas, and collision gas on the MS. Separation was carried out on a Zorbax Eclipse Plus C18 column (2.1 × 50 mm. 1.8 μm) with a C18 guard column (Agilent, USA).

2.3. Standard solutions

The stock standard solutions were prepared individually at a concentration of 100 μg/mL. Sudan I, Sudan II, Sudan Red G, Sudan Orange G, Sudan Red 7B, Dimethyl Yellow, Rhodamine B, Sudan Black B, Sudan Red B and Auramine O were prepared in ACN. Sudan III, Sudan IV, Para Red and Toluidine Red were dissolved in DCM, and Orange II was prepared in ethanol. An intermediate standard solution of the dyes at a concentration of 1000 ng /mL was prepared by dissolving a suitable volume of stock standard solutions in ACN. Standard solutions were stored in the in the dark at 4 °C.

2.4. Sample preparation and extraction

Tomato sauce, cotton candy, and cucumber pickle were obtained from the local market. A 1 g of homogenized sample was weighed into a 15 mL polypropylene centrifuge tube and extracted with 10 mL of ACN. Sample was then vortexed for 1 min and placed in an ultrasonic bath for 30 min. After sonication, sample was centrifuged at 7500 rpm (10 min, 15 °C) and 1 mL of supernatant was transferred to an LC autosampler vial. Sample vials were kept in the dark at 4 °C prior to analysis by LC-MS/ MS.

2.5. LC-MS/MS analysis

The mobile phase system consisted of A (10 mM ammonium formate in HPLC grade water with 0.1% formic acid) and B (10 mM ammonium formate in ACN with 0.1% formic acid). The linear gradient elution was as follows: 75% B (initial), 75% B (from 0 to 2 min), 98% B (2 to 3.50 min), 75% B (3.50 to 5 min). The flow rate was set at 500 μL/min, while the column temperature was maintained at 45 °C and the injection volume was 5 μL. Electrospray ionization mass spectra (ESI-MS) were acquired in the positive (ES +) and then negative (ESI−) ion mode. The Turbo Ion source operated at 500 °C with the electrode voltage at 5500 V. Other MS parameters were as follows: turbo gas, 50 psi; curtain gas, 25 psi; collision gas, 12 psi; entrance potential, 10 V. A total of 14 dyes – Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Red G, Sudan Orange G, Sudan Red 7B, Para Red, Dimethyl Yellow, Rahodamine B, Sudan Black B, Sudan Red B, Auramine O and Toluidine Red – were detected by positive ESI, and Orange II was detected by negative ESI. Dyes were identified by retention time and multiple reaction monitoring of each standard.

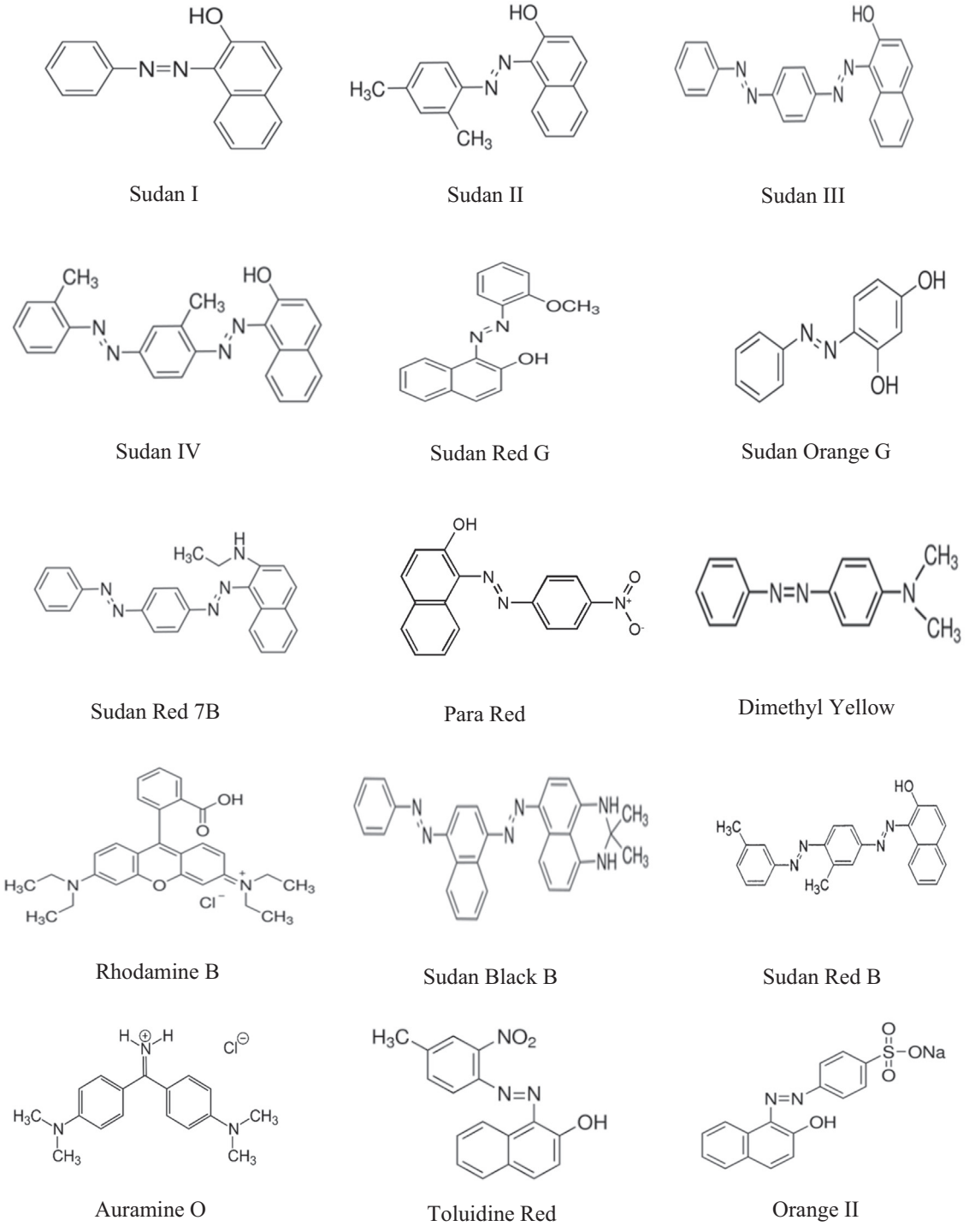

Fig. 1 Chemical structure of 15 dyes tested.

2.6. Validation study

To evaluate the suitability of the developed method to detect and quantify 15 synthetic dyes, the procedure specified by ISO/IEC 17025:2005 to validate non-standard methods was followed.

The specificity was evaluated by the analysis of 10 blank samples. The linearity was checked by analyzing 5 concentrations (1, 5, 10, 25 and 100 ng/mL) of all dyes in triplicate then, standard curves were generated. Furthermore, two concentrations (10 and 100 μg/kg) were spiked in blank sauce, cotton candy, and pickle, then analyzed 6 times to test for method precision, recovery and repeatability. On the next day, a different analyst repeated the same procedure by analyzing 4 different replicates of each matrix to test the reproducibility of the method. The recovery was determined based on mean measured values and expressed in percentage. The limit of detection (LOD) was calculated as three times the signal to noise ratio, while the limit of quantification (LOQ) was calculated as ten times the signal to noise ratio in the blank matrix.

3. Results and discussion

3.1. Optimization of LC-MS/MS conditions

Each dye was analyzed individually to determine precursor ion, product ion and retention time. Different combinations of mobile phase and elution conditions were experimented to achieve satisfactory optimal conditions in the minimal time possible. It was found that adequate separation can be reached in 5 min using Zorbax Eclipse Plus C18 column (2.1 × 50 mm. 1.8 µm) with a flow rate of 500 µL/min at 45 °C column temperature. Optimal conditions were assessed based on resolution and peak symmetry (Fig. 2). The addition of 10 mM ammonium formate in the mobile phase was found to facilitate the ionization of Orange II, but did not have any effect on other dyes. The declustering potential, entrance potential, and collision energy were set based on highest sensitivity yield (Table 1). Sensitivity of Orange II was found to increase in negative ESI, while all other dyes performed better in positive ESI.

3.2. Optimization of sample extraction

The use of SPE is costly and inconvenient for analysts when extracting large batches of samples. Therefore, the development of a practical and efficient extraction protocol to recover the maximum amount of synthetic dyes was necessary. It was found that ACN extraction coupled with sonication offers most reproducible results and highest recovers. ACN was chosen as an extraction solvent due to its low fat solubility and ability to precipitate proteins and carbohydrates. Another advantage this method adds is the small sample quantity, as the use of 1 g of material achieved good recovery. Overall, the recovery ranged from 82% to 119% for all synthetic dyes and the coefficient of variation of most data was below 15% (Table 2). Orange II was the only exception as recovery was 57% and 74% in cotton candy and pickle, respectively. The combination of high sugar content in cotton candy and the addition of formic acid in the mobile phase are probable causes for signal suppression, especially since Orange II was found to perform better in negative ESI compared to positive ESI. ACN: water mobile phase with ammonium formate in negative ESI are favorable conditions for glucose ionization (Thacker et al., 2018). Thus, Orange II signal was possibly suppressed by low access to droplet surface due to charge competition. Careful considerations must be taken when quantifying Orange II in high glucose matrices. However, for the purpose of this inclusive method the detection of Orange II combined with the good recovery for the other dyes was considered satisfactory.

3.3. Method validation

The linearity of calibration curves was assessed at the concentrations of 1, 5, 10, 25 and 100 µg/Kg for Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Red G, Sudan Orange G, Sudan Red 7B, Para Red, Dimethyl Yellow, Rahodamine B, Sudan Black B, Sudan Red B, Auramine O and Toluidine Red and Orange II. The regression equation and determination of the coefficient (R^2) of each dye are reported in (Table 3). Excellent linearities were achieved with correlation coefficients (R^2) > 0.996. The obtained LOD of dyes ranged from approxi-

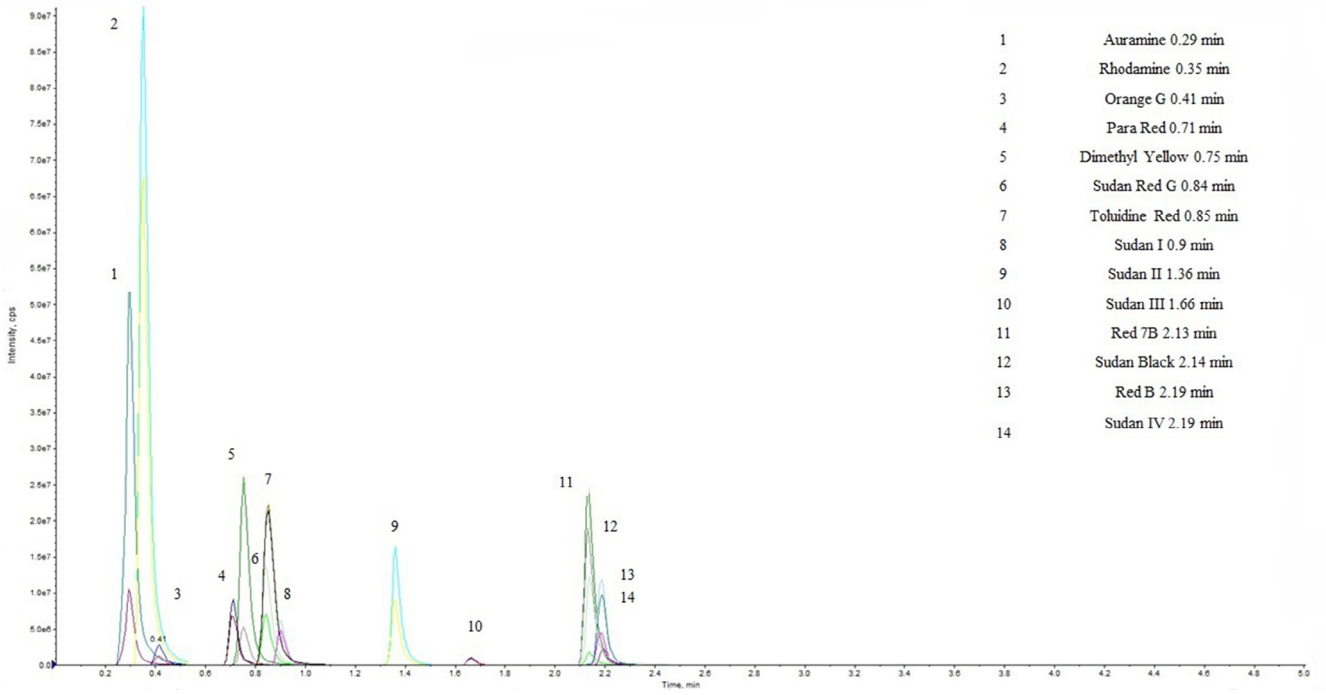

Fig. 2 Chromatogram of 15 dyes standard at 100 ng/mL (A) dyes detected in positive mode scan (B) dyes detected in negative mode scan.

Table 1 Tandem mass spectrometry acquisition parameters for the targeted synthetic dyes.

Analyte	Precursor ion (m/z)	Product ion (m/z)	Retention time (min)	Declustering potential (eV)	Entrance potential (eV)	Cell Exit Potential (CXP)
Sudan I	249.1	93	0.9	80	10	10
	249.1	156	0.9	80	10	11
Sudan II	277.2	121.1	1.36	60	10	11
	277.2	156.1	1.36	60	10	12
Sudan III	353.1	155.8	1.66	123	9	5
	353.1	92	1.66	123	9	8
Sudan IV	381.4	224	2.19	74	8.5	10
	381.4	276	2.19	74	8.5	10.5
Sudan Red G	279.1	123.1	0.84	70	10	11
	279.1	108.1	0.84	70	10	10
Sudan Orange G	215.1	93	0.42	73.5	8	5
	215.1	122	0.42	73.5	8	11
Sudan Red 7B	380.2	183.2	2.19	70	10	15
	380.2	115.1	2.19	70	10	15
Para Red	294.1	156.1	0.71	70	10	12
	294.1	128	0.71	70	10	10
Dimethyl Yellow	226.2	77.1	0.76	60	10	10
	226.2	121.1	0.76	60	10	10
Rhodamine B	443.1	399.1	0.35	61	10	9.02
	443.1	355.3	0.35	61	10	11.84
Sudan Black B	457.2	246.1	2.14	70	15	14
	457.2	142	2.14	70	15	8
Sudan Red B	381.3	224	2.19	68	8.5	12.73
	381.3	156	2.19	68	8.5	9
Auramine O	268.2	147	0.3	60	10	8
	268.2	122	0.3	60	10	8
Toludine Red	308.1	128	0.85	70	10	10
	308.1	152.1	0.85	70	10	11
Orange II	327	170.9	0.31	79–	5–	20–
	327	155.6	0.31	79–	5–	20–

Table 2 Mean recovery and coefficient of variation (n = 10) of individual dyes spiked at 2 levels (10 and 100 μg/kg) in sauce, cotton candy, and pickle.

Analyte	Recovery and coefficient of variation (CV) %					
	Sauce		Cotton Candy		Pickle	
	10 μg/kg	100 μg/kg	10 μg/kg	100 μg/kg	10 μg/kg	100 μg/kg
Sudan I	100.9 (2.5)	98.9 (0.8)	101.2 (3)	106 (2.2)	94.6 (2.7)	95.6 (2.2)
Sudan II	94.7 (5.5)	89.3 (3.2)	101.2 (4.9)	102.4 (7.3)	97.9 (6.1)	93.3 (4.9)
Sudan III	96.9 (3.8)	94.3 (3.2)	100 (2.1)	105.5 (4.3)	98.3 (2.4)	94.8 (9.5)
Sudan IV	101.5 (2.1)	100.1 (2.5)	102.6 (2.1)	104.3 (6.9)	98.8 (4.6)	100.5 (3.6)
Sudan Red G	102.6 (2.1)	98.5 (3.3)	101.8 (3.2)	103.4 (3.7)	98.4 (6.1)	93.9 (3.1)
Sudan Orange G	93 (4.8)	88.3 (3.1)	102.6 (3.4)	109.1 (2.7)	88.9 (9.5)	87.2 (6.8)
Sudan Red 7B	99.7 (6.6)	97.4 (1.9)	108.5 (4.1)	103.2 (3.6)	95.2 (9.3)	88.5 (17.6)
Para Red	100.6 (2.3)	97.7 (2.3)	92.9 (2.8)	104.4 (3.8)	94.4 (5.2)	93.2 (5.3)
Dimethyl Yellow	96.9 (4.3)	94.2 (3.9)	99.8 (3.7)	106.6 (1.8)	96.4 (5.9)	93.3 (4.7)
Rhodamine B	97.6 (4.6)	89.5 (4.1)	83.4 (4.7)	101.7 (3.1)	99.2 (5.7)	89.3 (2.9)
Sudan Black B	104.08 (10)	105.7 (3.6)	105.2 (5.7)	106.1 (4.4)	90.5 (11.5)	88.1 (17.6)
Sudan Red B	101.48 (1.6)	100.4 (3.7)	102.3 (2.3)	104.3 (6.7)	98.9 (4.5)	98.9 (4.3)
Auramine O	100.13 (5.8)	86.6 (3.9)	83.6 (7.3)	106.2 (6.2)	82.4 (17.4)	80.3 (5.2)
Toludine Red	110.8 (2.4)	99.9 (3.1)	107.9 (8.2)	103.6 (2.8)	102.5 (5.1)	95.5 (1.1)
Orange II	119.8 (3.8)	99.2 (9.6)	57.6 (19)	57.3 (16.8)	85.1 (15.3)	74.3 (13.1)

Table 3 Linear regression equation, linearity range and regression coefficients (R^2) of 15 synthetic dye.

Analyte	Linear range (ng/mL)	Linear equation	R^2
Sudan I	1–100	y = 160,000x + (–12,200)	0.9999
Sudan II	1–100	y = 407,000x + 162,000	0.9999
Sudan III	1–100	y = 29,400x + (–10,700)	0.9999
Sudan IV	1–100	y = 339,000x + (–359,000)	0.9998
Sudan Red G	1–100	y = 396,000x + 162,000	0.9997
Sudan Orange G	1–100	y = 80,000x + 12,000	0.9998
Sudan Red 7B	1–100	y = 727,000x + (–477,000)	0.9999
Para Red	1–100	y = 232,000x + (–328,000)	0.9996
Dimethyl Yellow	1–100	y = 744000x + 61,000	0.9999
Rhodamine B	1–100	y = 2,280,000x + 876,000	0.9999
Sudan Black B	1–100	y = 414,000x + (–283,000)	0.9999
Sudan Red B	1–100	y = 403,000x + (–433,000)	0.9998
Auramine O	1–100	y = 1,330,000x + 440,000	0.9997
Toludine Red	1–100	y = 676,000x + 1,520,000	0.9981
Orange II	1–100	y = 2200x + 21,400	0.9987

Table 4 Limit of detection (LOD) and limit of quantitation (LOQ) of 15 dyes in Sauce, Cotton Candy, and Pickle.

Analyte	Sauce		Cotton Candy		Pickle	
	LOD (μg/kg)	LOQ (μg/kg)	LOD (μg/kg)	LOQ (μg/kg)	LOD (μg/kg)	LOQ (μg/kg)
Sudan I	3.0	9.7	2.9	9.5	3.0	9.8
Sudan II	3.6	12.8	3.4	12.3	2.8	10.4
Sudan III	2.9	8.8	2.9	8.9	3.0	9.1
Sudan IV	2.8	6.95	1.8	3.6	3.0	7.5
Sudan Red G	3.5	12.7	4.1	14.6	2.8	10.4
Sudan Orange G	2.9	10.2	0.7	2.7	3.1	10.7
Sudan Red 7B	3.4	9.7	4.4	13.1	2.9	8.0
Para Red	4.1	10.5	4.2	10.8	3.4	8.0
Dimethyl Yellow	2.2	7.6	2.4	8.2	2.6	8.9
Rhodamine B	2.5	9.1	3.3	11.8	3.1	11.4
Sudan Black B	3.2	9.1	5	15	2.7	7.4
Sudan Red B	2.8	6.95	3.5	9.2	3.0	7.4
Auramine O	1.4	5.3	4.4	15.4	2.9	10.5
Toludine Red	1.9	11.5	2.6	14	1.8	11.1
Orange II	5.4	18.0	5.0	16.6	1.5	5.0

mately 0.7 to 5 μg/kg, whereas the LOQ was found to range from 7 to 15 μg/kg (Table 4). The only exception was Orange II, as LOQ was relatively higher in sauce and cotton candy due to high background noise. Overall, LOD and LOQ of all included dyes are much lower than conventional HPLC-DAD and HPLC -UV methods. Additionally, Sudan III, Sudan IV, Sudan Orange G and Sudan Red B LOD and LOQ significantly improved using Qtrap mass analyzer compared to the triple quadrupole used by Tsai et al. (2015).

3.4. Proficiency testing

To test the reliability of the developed method, the laboratory participated in a proficiency test provided by Fapas (York, UK) to quantify Sudan I, Sudan Red G, Para Red and Toluidine Red in hot pepper sauce (T20132QC). The method successfully passed the proficiency test with a Z-score ≤ 2 and recovery range between 93 and 104% for all analyzed dyes (Table 5).

Table 5 Fapas proficiency test results.

Analyte	Assigned value (μg/kg)	Ranged of I Z I ≤ 2	Obtained value (μg/kg)	Recovery (%)
Sudan I	381	240–522	363	95.2
Sudan Red G	484	311–657	451	93.1
Para Red	962	653–1272	970	100.8
Toludine Red	250	152–349	260	104

4. Conclusion

The essence of this study was to create a practical method to analyze for food colorant that can be applied to support large scale food additives surveillance programs. Using a simple one step extraction, an LC/MS-MS method was developed to simultaneous detect and quantify 15 banned dyes in sauce, cotton candy, and pickle. The preparation procedure requires only 1 g of material and has shown to have excellent recovery. The newly developed method has been validated using the criteria specified by ISO/IEC 17025:2005. The selection of two fragment ion transitions provides high sensitivity and accuracy. Additionally, the method has good repeatability, excellent recoveries with low detection limits; and the proficiency test results demonstrate its reliability. In future work, it is possible to widen the scope to include other dyes and investigate the effect of different matrices.

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Declaration of Competing Interest

The authors declare that no competing interests exist in relation to this manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2019.09.008.

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