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Quantitative analysis of illegal dyes in eggs using LC/MS/MS

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Introduction

Generally, egg yolk color is an indication of its nutritional value and freshness. Eggs with yellow-orange hue are the most desired ones. Hence, egg producers enhance the color of their products by feeding the egg laying hens with feed containing various synthetic dyes (shown in Figures 1 and 2). This can be proved by confirming the presence of illegal dye residues in eggs[1].

Figure 1. Duck eggs

Figure 2. sudan dyes in egg yolk

A method for pre-treatment and subsequent LC/MS/MS analysis has been developed for highly sensitive quantitation of these dyes from egg using LCMS-8040, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan.
Ultra-fast scanning speed of LCMS-8040 enables simultaneous analysis of four sudan dyes and para red dye. All these dyes were quantitated at low ppb levels with high specificity and good repeatability even in presence of egg matrix.
Sudan and para red dyes consists of a group of synthetic industrial dyes like sudan I, II, III and IV etc. The International Agency for Research on Cancer evaluated the safety of sudan I, II, III and IV in 1987 and considered that they were unclassifiable as to their carcinogenicity to humans. Although there is some evidence that sudan dyes may cause cancer in experimental animals and may cause damage to the genes, there is currently inadequate evidence that they cause cancer in humans.
Structure of four sudan dyes and para red under study are as shown in Figure 3.

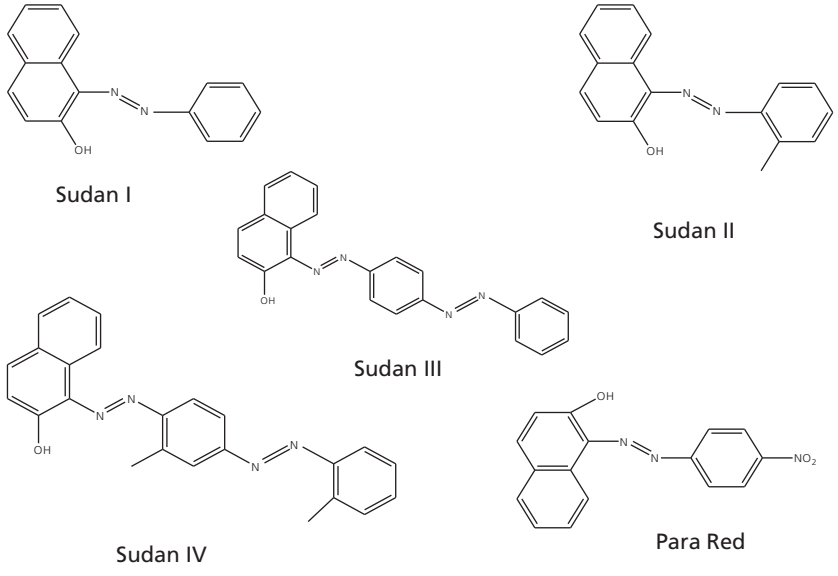

Figure 3. Structure of sudan dyes and para red used to contaminate egg

Method of analysis

Preparation of matrix matched calibration curve

In 1 g of egg yolk, 9 mL water and 10 mL acetonitrile was added in 50 mL tarson tubes. Mixed it well for one min. For extraction, QuEChERS Kit [2] (1 packet EN 15662) (Phenomenex Inc.) was added into it and mixed thoroughly for one min. Centrifuged these tubes at 4000 rpm for 5 min at -10 °C and collected approximately 8 mL of supernatant.
Passed the 3 mL of collected supernatant through a preconditioned C-18 SPE cartridge (Orochem), which finally eluted with 3 mL acetonitrile. This eluent was used to prepare dilutions for matrix matched calibration in range of 1 ppb - 100 ppb.

LC/MS/MS analysis

Compounds were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8040 triple quadrupole system (Shimadzu Corporation, Japan). The details of analytical conditions are given in Table 1.
LCMS-8040 triple quadrupole mass spectrometer as shown in Figure 4, with its ultrafast polarity switching and ultrafast MRM, along with high sensitivity is best suited instrument for food safety analysis. These features ensure analysis of multiple components with different ionizing tendency to be analyzed within same run, without any loss of sensitivity.

Figure 4. Nexera with LCMS-8040 triple quadrupole system by Shimadzu

Table 1. LC/MS/MS conditions for analysis of sudan dyes and para red

Column	: Shim-pack XR-C8 (75 mm L x 3 mm I.D.; 2.2 µm)
Flow rate	: 0.4 mL/min
Oven temperature	: 40 °C
Mobile phase	: A: 0.1% formic acid in water
	B: 0.1% formic acid in acetonitrile
Gradient program (B%)	: 0.01 – 1.50 min → 50 - 80 (%); 1.50 – 2.50 min → 80 (%);
	2.50 – 4.0 min → 80 - 100 (%); 4.0– 6.0 min → 100 (%);
	6.0 – 6.1 min → 100 - 50 (%); 6.1 – 10.0 min → 50 (%)
Injection volume	: 20 µL
MS interface	: Electro Spray Ionization (ESI)
Nitrogen gas flow	: Nebulizing gas 3 L/min; Drying gas 10 L/min
MS temperature	: Desolvation line 230 °C; Heating block 400 °C
MRM Transitions	: Sudan I: 249.00>93.20
	Sudan II: 277.00>121.15
	Sudan III: 353.00>77.10
	Sudan IV: 381.00>225.20
	Para red: 294.20>156.10

Results

Analysis of sudan and para red dyes was carried out in both aqueous and egg yolk matrix. MRM was optimized using automatic MRM optimization feature of LabSolutions. Analysis was also checked for blank interference. The method optimized had substantially reduced background interferences.

Result of simultaneous analysis of five dyes in egg yolk is shown in Figure 5. Overlay of blank, LOQ and highest concentrations are shown in Figure 6. Linearity and accuracy were checked for both aqueous and matrix matched calibration. These quantitative results are given in Table 2. LOQ of 1 ppb was obtained for all the five dyes in both aqueous and egg yolk matrix.

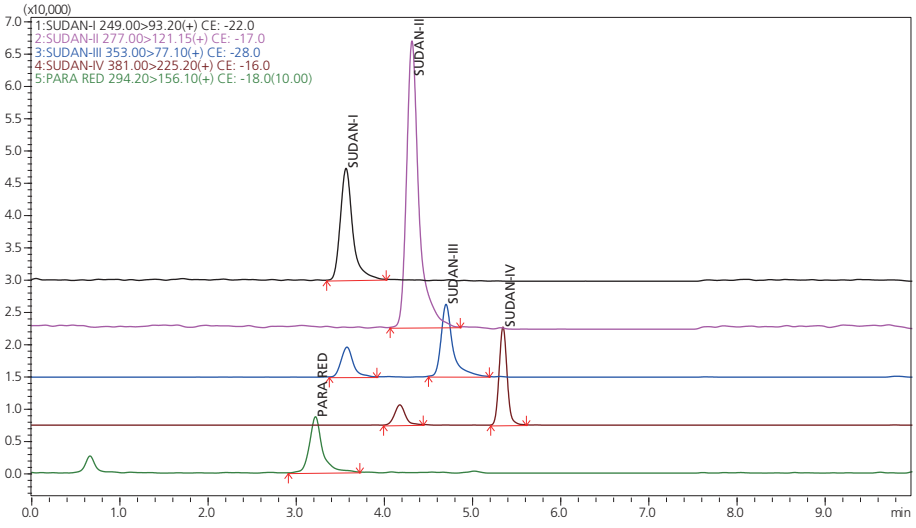

Figure 5. Overlay of sudan dyes and para red for 5 ppb standard in egg yolk

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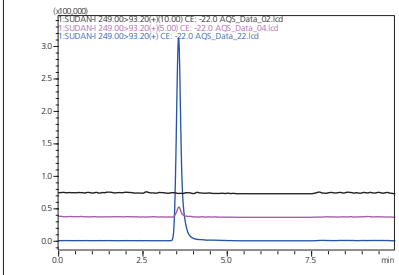
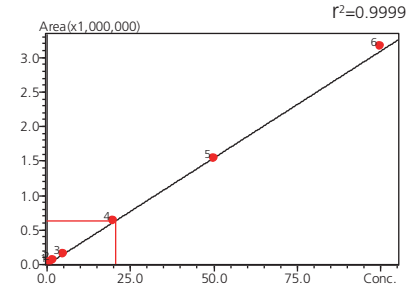
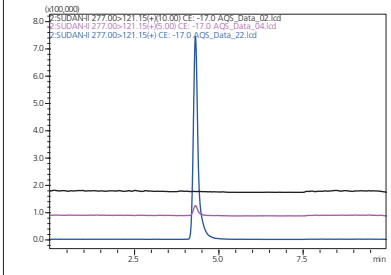
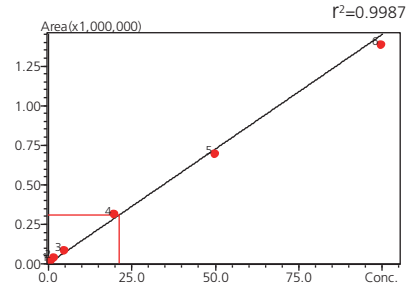
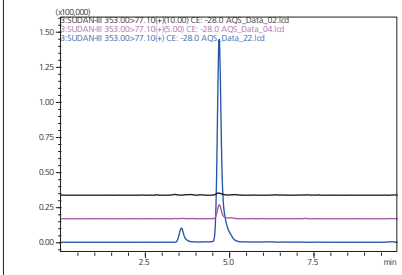
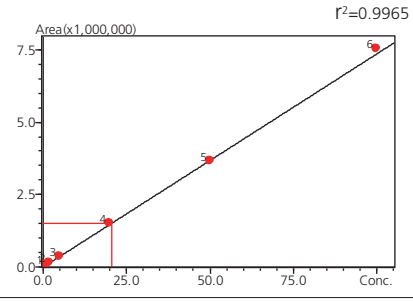
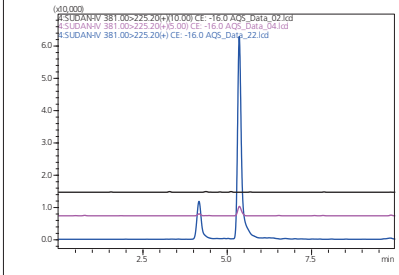
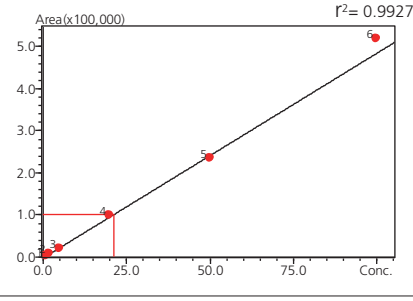
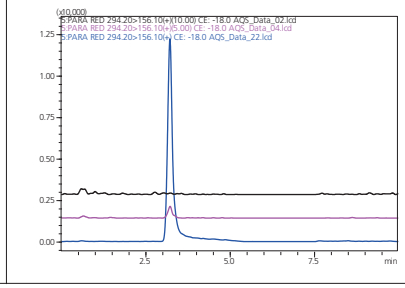
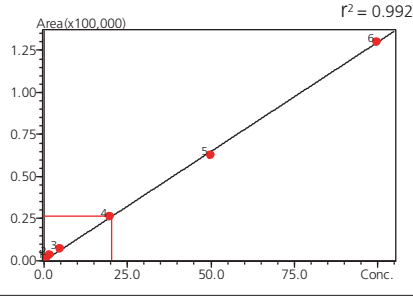
Name	Overlay of matrix blank, 1 ppb, 100 ppb	Calibration curve (1 ppb – 100 ppb)
Sudan I		
Sudan II		
Sudan III		
Sudan IV		
Para red		

—— Matrix blank; —— 1 ppb; —— 100 ppb

Figure 6. Overlay mass chromatograms and calibration curve for sudan dyes and para red in egg yolk matrix

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Table 2. Results of sudan dyes and para red calibration curve

Name	Nominal concentration (ppb)	Measured concentration* (ppb)	Avg. area*	%RSD cal.point area*	% accuracy*
Sudan I	1	1.0	35597	7.45	100.4
	2	1.9	69150	0.86	99.2
	5	4.9	172070	3.59	99.8
	20	19.9	684163	1.62	99.7
	50	50.3	1722867	0.70	100.6
	100	100.1	3426385	0.65	100.1
Sudan II	1	0.9	79040	2.74	97.6
	2	2.1	176546	1.75	105.7
	5	5.0	428791	2.47	101.2
	20	19.6	1677856	2.20	98.2
	50	49.1	4199978	0.83	98.2
	100	99.8	8537248	1.07	99.7
Sudan III	1	0.9	21571	2.09	98.2
	2	2.0	41915	9.33	100.3
	5	5.3	109039	2.54	107.7
	20	20.7	413989	2.84	103.7
	50	49.6	987767	1.21	99.3
	100	93.0	1848722	1.48	93.0
Sudan IV	1	1.0	20996	4.96	106.9
	2	2.2	38519	1.36	111.3
	5	5.6	91127	1.21	113.3
	20	19.9	307351	0.98	99.8
	50	50.8	774778	1.20	101.6
	100	91.2	1387058	2.02	91.2
Para red	1	1.0	1605	7.70	100.1
	2	1.9	3256	9.29	99.2
	5	5.1	8498	5.48	102.1
	20	19.6	32836	3.55	98.1
	50	49.8	83635	1.88	99.8
	100	100.9	169136	1.75	100.8

* Average result for n=3

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Conclusion

- Highly sensitive method for simultaneous analysis of illegal dyes in egg yolk like four sudan dyes and para red was developed.
- LOQ of 1 ppb was obtained for all five dyes by matrix matched calibration standards, with linearity > 0.99 and accuracy between 90-110%.
- An analytical method was developed to minimize the background interference, hence giving high level of sensitivity.

References

[1] Marta Piatkowska, Piotr Jedziniak, Jan Zmudzki, Bull Vet Inst Pulaway, Volume 58, (2014), 247- 253.
[2] Javier Dominguez- Alvarez et al., Journal of Chromatography A, Volume 1278, (2013), 166- 174.

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