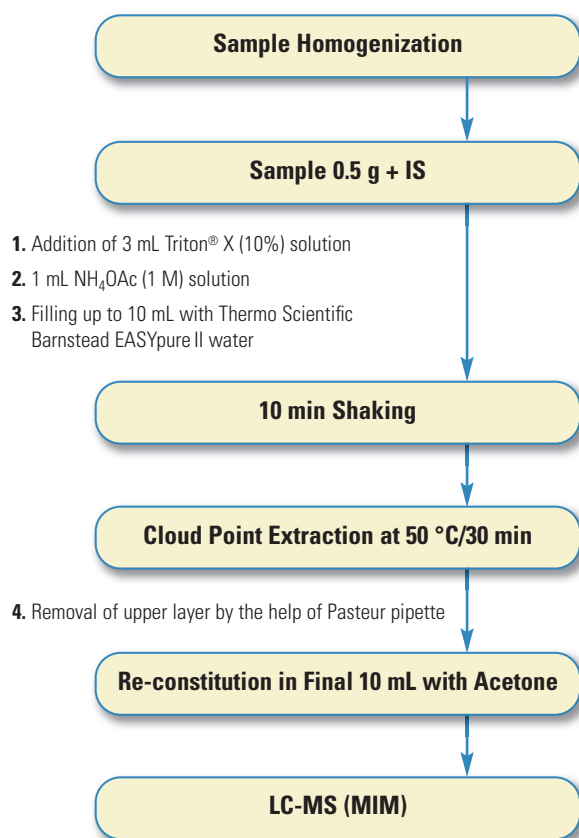# LC-MS Method Using Cloud Point Extraction for the Determination of Permitted and Banned Azo Colors in Liquid, Semi-liquid and Solid Food Matrices

Ebru Ates, Klaus Mittendorf, Thermo Fisher Scientific, Dreieich, Germany

1. Schematic of Method

```
              Sample Homogenization
                       │
                Sample 0.5 g + IS
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1. Addition of 3 mL Triton® X (10%) solution

2. 1 mL NH_4OAc (1 M) solution

3. Filling up to 10 mL with Thermo Scientific Barnstead EASYpure II water

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                  10 min Shaking
                       │
        Cloud Point Extraction at 50 °C/30 min
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4. Removal of upper layer by the help of Pasteur pipette

```
        Re-constitution in Final 10 mL with Acetone
                       │
                   LC-MS (MIM)
```

Although there are many methods for the determination of Sudan I-IV, there is no single method for ascertaining a number of different types of colors in a variety of food matrices.

Cloud Point Extraction (CPE), compared to solvent extraction is a particularly attractive option employing non-ionic surfactants which provide effective, cheap, easy and environmentally friendly extraction and pre-concentration. In this Method, we describe a new CPE method for determining the presence of approved water soluble (sunset yellow, allura red, tartrazine and erythrosine) and banned fat soluble (Sudan I-IV, Sudan red G, Sudan red B, Sudan red 7B, Sudan black B, metanil yellow and rhodamine B) azo colors, followed by separation on a Thermo Scientific Hypersil GOLD column and detection with a Thermo Scientific MSQ Plus single quadrupole mass spectrometer. This method has been validated using liquid (home-made wine), semi-liquid (home-made paprika sauce) and solid (home-made chili) food and beverages. With matrix-matched calibration in representative blank foodstuff for each matrix linearity, specificity, recovery, inter- and intra- day repeatability and robustness of the method were established.

2. Introduction

Colors are food additives that are regulated in the EU[1], USA[2], and China[3]. Only approved food colors can be used and others are banned such as some azo compounds, because of their potential carcinogenicity. The so-called Sudan colors are usually used for coloring oils or as additives in shoe polish, but have been used illegally in foods. They are classified as class 3 carcinogens by the International Agency for Research on Cancer (IARC).[4] Despite considerable publicity these banned colors are still found in foodstuffs, e.g. chili and chili containing products, according to recent RASFF alerts.[5] Directive 2005/402/EC introduced emergency measures that has required certification of each product exported to the EU to be certified to be free of Sudan colors.[6]

3. Scope

This method for regulated water-soluble and banned fat-soluble colors can be applied to liquid, semi-liquid and solid food samples. The method gives a quantitative indication of banned azo colors (Sudan I-IV, Sudan red G, Sudan red B, Sudan red 7B, Sudan black B, metanil yellow and rhodamine B), at an LOD of 0.5 mg/kg which EU Member States agreed as an action limit. Simultaneously, regulated food colors (sunset yellow, allura red, tartrazine and erythrosine) can be analyzed in liquid, semi-liquid and solid food and beverages.

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4. Principle

The homogenized food sample is fortified with an appropriate labeled internal standard. After addition of aqueous surfactant (Triton X-114) and ammonium acetate solution, the sample is shaken and heated in a water bath. After Cloud Point Extraction the upper aqueous layer is removed with a pasteur pipette. The surfactant rich layer is diluted and analyzed by LC-MS using multi ion monitoring (MIM).

5. Reagent List

		Fisher Scientific USA Part Number
5.1	Triton X-114	T/3757/08
5.2	Acetone, HPLC Grade	A/0606/17
5.3	Acetonitrile, LC-MS Grade	A/0627/17
5.4	Water, LC-MS Grade	W/0112/17
5.5	Methanol, HPLC Grade	M/4058/17
5.6	Ammonium acetate	BP326-500
5.7	Ethyl acetate	E/0906/17
5.8	Formic acid	J/4200/PB08
5.9	Ethanol, HPLC Grade	E/0665DF/17

6. Calibration Standards

6.1	Metanil Yellow	Fisher Scientific
6.2	Rhodamine B	Fisher Scientific
6.3	Sudan Black B	Dr. Ehrenstorfer
6.4	Sudan I	Sigma-Aldrich
6.5	Sudan II	Sigma-Aldrich
6.6	Sudan III	Dr. Ehrenstorfer
6.7	Sudan IV	Sigma-Aldrich
6.8	Sudan Orange G	Sigma-Aldrich
6.9	Sudan Red 7B	Sigma-Aldrich
6.10	Sudan Red B	Dr. Ehrenstorfer
6.11	Sudan Red G	Sigma-Aldrich
6.12	Allura Red	Sigma-Aldrich
6.13	Erythrosine	Sigma-Aldrich
6.14	Sunset Yellow	Sigma-Aldrich
6.15	Tartrazine	Sigma-Aldrich
Internal Standard		
6.16	d_6 Sudan IV	Dr. Ehrenstorfer

7. Standards Preparation

7.1 Stock standard solutions of fat soluble colors (1 mg/mL)

Prepare stock solutions of fat soluble colors in the appropriate solvent listed in 7.1.1. Weigh 10 mg (except Sudan red 7B with 5 mg) of color into a 10 mL volumetric flask and fill up to the mark with the appropriate solvent. Place stock solutions in an ultrasonic bath for 5 min to dissolve. Store standard stock solutions in amber flasks. The solutions must be handled with care to avoid exposure to light.

7.1.1 Concentrations for stock solutions of fat soluble colors in corresponding solvents

Metanil Yellow:	1 mg/mL methanol
Rhodamine B:	1 mg/mL methanol
Sudan Black B:	1 mg/mL ethyl acetate
Sudan I:	1 mg/mL methanol
Sudan II:	1 mg/mL ethyl acetate
Sudan III:	1 mg/mL ethyl acetate
Sudan IV:	1 mg/mL ethyl acetate
Sudan Red 7B:	0.5 mg/mL methanol
Sudan Red B:	1 mg/mL ethyl acetate
Sudan Red G:	1 mg/mL ethyl acetate

7.2 Working standard solution of fat soluble colors

Prepare a standard mixture of fat soluble colors in acetone with a final concentration of each color of 0.01 mg/mL. Prepare working standard mixture freshly every week and store in an amber flask. The solution must be handled with care to avoid any light.

7.3 Stock standard solutions of water soluble colors

Prepare stock solutions of water soluble colors in water. Weigh 10 mg of color into a 10 mL volumetric flask and fill to the mark with water (LC-MS Grade). Place stock solutions in an ultrasonic for bath 5 min to dissolve. Store the standard stock solutions in amber flasks. The solutions must be handled with care to avoid any exposure to light.

7.3.1 Concentrations for stock solutions of water soluble colors

Allura Red:	1 mg/mL water
Sunset Yellow:	1 mg/mL water
Erythrosine:	1 mg/mL water
Tartrazine:	1 mg/mL water

7.4 Working standard solution of water soluble colors

Prepare working standard mixture of water soluble colors in water with a final concentration of each color of 0.1 mg/mL, except erythrosine which is 0.15 mg/mL. Prepare working standard mixture freshly every week and store in an amber flask. The solution must be handled with care to avoid exposure to light.

7.5 Stock standard solution of internal Standard

Prepare a stock solution of d_6-Sudan IV as internal Standard in ethyl acetate. Weigh 10 mg of color into a 10 mL volumetric flask and fill to the mark with ethyl acetate. Place for 5 min the stock solution in an ultrasonic bath to dissolve. Standard stock solutions must be stored in amber flasks. The solutions must be handled with care to avoid exposure to light.

7.6 Working standard solution of internal Standard

Prepare a working standard mixture of d_6-Sudan IV as internal standard at a final concentration of 0.01 mg/mL in acetone. Prepare a working standard mixture freshly every week and store in an amber flask. The solution must be handled with care to avoid exposure to light.

7.7 Reagents

10% Triton X-114:	100 g Triton X-114 in 9900 mL of water (EASYpure™ II), after weighing stir the mixture with a magnetic stirrer
1 M Ammonium acetate:	7.7 g/100 mL water (EASYpure II), after weighing stir the mixture with a magnetic stirrer

8. Apparatus

		Fisher Scientific USA Part Number
8.1	MSQ™ Plus Single Quadrupole LC-MS	40000
8.2	Accela Autosampler	60057-60020
8.3	Accela Pump 1250	60057-60111
8.4	Fisher precision balance	XP-1500FR
8.5	Sartorius® analytical balance	ME235S
8.6	EASYpure II water	3125753
8.7	Ultrasonic bath Elmsonic S40H	1002006
8.8	ULTRA-TURRAX® – dispergation tool	1713300
8.9	ULTRA-TURRAX – Plug-in coupling	1024200
8.10	ULTRA-TURRAX	3565000
8.11	Vortex shaker	3205025
8.12	Vortex standard cap	3205026
8.13	Büchi®, heater bath	3214576
8.14	IKA® HS 501, digital Shaker	9838041
8.15	IKA HS 501, universal holder part	9838046
8.16	Fisherbrand, magnetic stir bar	9662340
8.17	RCT basic IKAMAG®, magnetic stirrer	3203725
8.18	Centrifuge tube rack	919 45 35

9. Consumables

		Fisher Scientific USA Part Number
9.1	Hypersil GOLD™, 150 × 2.1 mm 1.9 µm Thermo Scientific P/N 25002-152130	3212496
9.2	Centrifuge tubes, 15 mL	FB55951
9.3	LC vials	3205111
9.4	LC vial caps	3151266
9.5	PTFE Filter, 0.2 µm	3152906
9.6	1 mL syringe	9410000
9.7	Pipette, Finnpipette 10–100 µL	3166472
9.8	Pipette, Finnpipette 100–1000 µL	3214535
9.9	Pipette, Finnpipette 500–5000 µL	3166473
9.10	Pipette holder	3651211
9.11	Pipette, Pasteur soda lime glass 150 mm	FB50251
9.12	Pipette suction device	3120891
9.13	Pipette tips 0.5–250 µL, 500/box	3270399
9.14	Pipette tips 1–5 mL, 75/box	3270420
9.15	Pipette tips 100–1000 µL, 200/box	3270410
9.16	Spatula, 18/10 steel	3458179
9.17	Spatula, nylon	3047217
9.18	Wash bottle, PTFE	3149330

10. Glassware

		Fisher Scientific USA Part Number
10.1	Fisherbrand, Beaker, 25 mL	9653010
10.2	Fisherbrand, Pasteur pipette	FB50251
10.3	Fisherbrand, Volumetric flask, 10 mL	FB50143
10.4	Fisherbrand, Volumetric flask, 100 mL	FB50151
10.5	Fisherbrand, Volumetric flask, 1000 mL	FB50159

11. Procedure

11.1 Sample preparation

Liquid Matrix

11.1.1 Weigh 0.5 g of the liquid (wine, bitter soda or flavored milk drink) into a 15 mL disposable polypropylene centrifuge tube and add 125 µL of working internal Standard solution.

Semi-liquid Matrix

11.1.2 Homogenize 200 g of semi-liquid matrix (sauces, soups or pastes) in a high speed blender for 5 min. Weigh 0.5 g into a 15 mL disposable polypropylene tube and add 125 µL of internal standard solution.

Solid Matrix (Insoluble)

11.1.3 Homogenize 100 g of solid matrix (spices, seasonings or chilis) in a high speed blender for 5 min. Weigh 0.5 g into a 15 mL disposable polypropylene tube and add 125 µL of working internal standard solution.

Blank Matrixes

For calibration purposes, use blank foodstuffs representative of each of the respective matrix types.

Liquid matrix (sodas, juices, wine and flavored milk drinks) comprising a self prepared wine (homogenized, filtered 1 kg grape including 10% ethanol) was used as blank material.

Semi-liquid matrix (mainly representing sauces and pastes) comprising home-made pure paprika sauce.

Solid matrix (mainly representing seasonings and spices) comprising home-made chili powder (500 g of red and yellow paprika was cut, dried (40 °C/24 h) and homogenized in a high speed blender).

11.2 Cloud Point Extraction

11.2.1 Add 3 mL of the prepared Triton X-114 solution (7.7) and 1 mL of ammonium acetate solution (7.7) to the sample fill up to 10 mL with water and shake for 10 min in the horizontal shaker.

11.2.2 Put the samples for 30 min in a 50 °C water bath. As samples get cloudy, phase separation occurs.

11.2.3 Wait 5-10 min for the samples to cool at room temperature to achieve better phase separation

11.2.4 Remove the upper water layer with a Pasteur pipette

11.2.5 Reconstitute the extract in a final volume of 10 mL acetone

11.2.6 Filter the extract with a PTFE filter 0.2 µm into a LC-vial and inject to the LC-MS-system

11.3 Detection

11.3.1 LC operating conditions

LC analysis was performed on a Thermo Scientific Accela AS and Accela 1250 Pump system (Thermo Fisher Scientific, San Jose, CA USA). The LC conditions were as follows:

Column: Hypersil GOLD, 150 × 2.1 mm, 1.9 µm
Injection Volume: 10 µL
Injection Mode: partial loop
Tray Temperature Control: 22 °C
Column Oven Temperature: 22 °C
Mobile Phase: A: water (0.1% formic acid);
　　　　　　 B: acetonitrile (0.1% formic acid)

Gradient:

Time	A [%]	B [%]
0	99	1
5	99	1
10	30	70
15	25	75
35	25	75
35.01	99	1
42	99	1

11.3.2 Mass spectrometric conditions

MS analysis is carried out using a MSQ Plus LC single quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA USA). Data acquisition and processing was performed using Thermo Scientific Xcalibur 2.1 software. The MS conditions were as follows:

Ionization: Electrospray (ESI)
Polarity: positive negative switching
Probe Temperature: 450 °C
Cone Voltage: 150 V
Scan Mode: multi ion monitoring (MIM)
ESI Voltage: 3.0 kV

Program the mass spectrometer with 16 timed segments which is set-up for the 14 target colors including respective labeled internal standard. The program of segments for MIM events is shown in Table 1.

12. Calculation of Results

12.1 Identification

Identification of colors was indicated by the presence of at least two characteristic ions measured in multi-ion monitoring mode (MIM) at the corresponding retention time to those of the respective standards shown in Table 1. Light induced isomers of Sudan black B show two peaks, which is the reason for two timed segments for detection of this analyte (Table 1).

12.2 Quantification

For quantification, compare the peak areas of the colors in the spiked samples with those of matrix-matched standard solution. Prepare these samples and matrix-matched calibration curve by spiking an aliquot (0.5 g) of blank matrix with the desired amount of standard solution.

Carry out internal standardization using peak area ratios for standards in matched matrices. Use d_6- Sudan IV as the internal standard for all colors. Plot the calibration curves as the relative peak areas (analyte versus the corresponding internal standard) as a function of the compound concentration. Determine the color concentration (C_{Color}) in the samples from the equation:

$$C_{Color} = \left(\frac{A_{Color}}{A_{[IS]}}\right) - b/a$$

where,

C_{Color} – color concentration in mg/kg

A_{Color} – peak area of the color

$A_{[IS]}$ – peak area of internal standard

b – the y-intercept

a – the slope of calibration curve

Dilute any samples initially found to contain levels of colors outside the linear range, and take the dilution factor into account in the final calculations.

13. Method Validation

In-house validation method performance characteristics were established by spiking experiments with blank samples [self prepared wine, home-made paprika paste and home-made dried chili (see. 11.1)] with a mixture of fat soluble and water soluble color standards, except only spiking with water soluble colors in wine.

Method accuracy was determined at three different spiking levels of colors [low (around LOQ), mid (expected concentration levels) and high level]. Intermediate precision during three day sample preparation of mid level value was prepared. Other validation parameters included determination of linearity range, limits of detection/quantification, robustness and matrix effects.

13.1 Specificity

Using multi-ion monitoring (MIM) the specificity was confirmed based on the presence of fragment ions at the correct retention time corresponding to the color standards in solvent. According to 657/2002 EC the deviation for retention time should fall within the range of ±2.5% in all matrices.[7]

13.2 Linearity and Calibration Curve

The linearity of calibration curves was assessed over the range from 0.3–25 mg/kg (for fat soluble colors), from 20–300 mg/kg (for water soluble colors) and 30–450 mg/kg for erythrosine. In all cases, the correlation coefficients of linear functions should be >0.985. The calibration curves were created from five matrix-matched calibration standards which are injected in each batch in duplicate.

13.3 Accuracy

Method accuracy and precision (Table 2 and Table 3) was assessed by recovery studies using blank matrices of the studied matrices (wine, sauce and chili) spiked at three concentration levels. Samples were spiked at 1.5, 5 and 15 mg/kg (fat soluble colors) and 50, 150 and 300 mg/kg (water soluble colors and 75, 225 and 450 mg/kg for erythrosine) levels.

Found concentrations (mg/kg), recovery, standard deviation (STDV) and relative standard deviation (% RSD) were calculated. Matrixes described in 11.1 were used for matrix matched calibration as representative blank matrices. RSD values between 1.6–16.9% are acceptable for a regulatory method (<20%). Recovery results are within the regulatory range of 60–120% except sunset yellow and allura red in wine (Table 2).

13.4 Intra- and Inter-day Precision

Intra- and inter- day precision was established for each matrix by spiking (blank wine, blank sauce and blank chili) at the same level (mid level) with 6 replicates. Three different batches are prepared and the RSD values of intra- and inter day compared. Table 3 shows intra- and inter- day RSD values ranging from 3.6–13.9% as intra- and 5.9–19.1% as inter day precision values respectively.

13.5 Limits of Detection (LOD) and Quantification (LOQ)

Limits of detection and quantification were estimated following the IUPAC approach which consisted of analyzing the blank sample to establish noise levels and then estimating LODs and LOQs for signal/noise, 3 and 10 respectively. The LOQ and LOD values are listed in Table 4. In all cases (except sunset yellow in flavored milk products (regulated limit 50 mg/kg), these values exceed requirements to test for compliance to regulatory limits for which 0.5 mg/kg (LOD) is set as lowest level which is controlled.

13.6 Robustness

The Robustness of the method was determined by using another supplier of Triton X-114; by varying the shaking time instead of 10 min to 5 and 15 min; by changing the extraction time from 30 to 40 and 50 min and by changing the extraction temperature from 50 to 60 and 70 °C during determination of inter day precision (Table 3).

14. Conclusion

This method allows fast, cheap and environmentally friendly determination of 14 colors from liquid (wine, soft drinks and flavored milk drinks), semi-liquid (sauces, pastes and soups) and solid (chilies, spices and seasoning) matrices. The method performance indicates it is suitable for routine use for regulatory purposes.

15. Acknowledgement

16. References

1. Commission Decision 1994/36/EC of 30 June 1994 on colors for foodstuffs. Official Journal of the European communities, L 237/13, **1994**.

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4. IARC. Monographs on the evaluation of the carcinogenic risk of chemicals to man: some aromatic azo compounds (Vol. 8). Lyon, France: International Agency for Research on Cancer, 224-231, **1975**.

5. The Rapid Alert System for Food and Feed (RASFF). Annual report **2009**.

6. Commission Decision 2005/402/EC of 23 May 2005 on emergency measures regarding chili, chili products curcuma and palm oil. Official journal of the European communities, L135, 34, 36, **2005**.

7. Commission Decision 657/2002 of August 2002 on implementing Council Directive 96/23/EC concerning performance of analytical methods and the interpretation of results. Official journal of the European communities, L221, 8-36, **2002**.

17. Annex

17.1 Tables and Chromatograms

Compound	Time Range (min)	M (g/mol)	Ion 1	Ion 2	Ion 3	Ion 4	Dwell Time	Polarity
Allura Red	6-10	450.94	136.40	206.50	450.94	–	0.02	-
Sunset Yellow	6-10	407.01	142.35	206.55	142.35	–	0.08	-
Erythrosine	9-15	835.31	833.89	834.63	835.31	–	0.03	-
Tartrazine	15-20	470.87	469.03	470.05	470.87	–	0.08	+
Metanil Yellow	9-12	354.22	100.12	117.32	354.22	–	0.04	+
Rhodamine B	8.5-13	443.10	443.09	415.15	399.09	–	0.04	+
Sudan I	13-16.5	249.37	168.83	231.45	249.37	–	0.08	+
Sudan II	16-21	277.45	169.03	118.05	277.45	–	0.1	+
Sudan III	19.5-23	353.15	118.31	156.1	353.16	–	0.04	+
Sudan IV	28-34	381.17	132.39	168.91	381.17	–	0.2	+
Sudan Red G	12-17	279.33	79.75	279.33	–	–	0.08	+
Sudan Red B	28-34.5	381.18	156.16	225.53	381.19	–	0.5	+
Sudan Red 7B	29-35	380.18	168.79	183.59	380.19	–	0.15	+
Sudan Black B 1st	17.5-21.5	457.09	211.81	247.48	287.26	457.09	0.2	+
Sudan Black B 2nd	30-37	457.09	211.81	247.48	287.26	457.09	0.2	+
Sudan IV d$_6$	27-33	389.20	387.2	388.16	389.20	–	0.5	+

Table 1: Detection conditions of chromatographic method by multi ion mode (MIM) with HPLC-ESI$_{+/-}$-MS

Color	Mean Recoveries (%)				Mean Relative Standard Deviation (RSD %)		
	Wine	Sauce	Chili		Wine	Sauce	Chili
Sunset Yellow	52.4	99.7	84.9		4.0	9.0	4.9
Allura Red	50.4	69.6	82.1		4.7	14.0	11.2
Tartrazine	**	100.3	**		**	5.3	**
Erythrosine	105.3	111.6	104.4		6.8	14.8	13.1
Metanil Yellow	*	116.0	112.4		*	7.6	5.4
Rhodamine B	*	115.6	98.9		*	10.7	8.2
Sudan I	*	101.7	111.9		*	10.3	6.0
Sudan II	*	109.7	107.7		*	8.4	4.9
Sudan III	*	104.5	97.4		*	10.3	8.1
Sudan IV	*	105.9	102.3		*	6.4	4.2
Sudan Red B	*	78.7	100.6		*	10.7	8.2
Sudan Red 7B	*	81.3	111.8		*	9.6	6.9
Sudan Red G	*	107.3	101.1		*	10.4	5.9
Sudan Black B 1st	*	102.9	113.1		*	13.9	6.6
Sudan Black B 2nd	*	100.8	110.1		*	5.9	5.7

Table 2: Mean Recovery (%) and Mean RSD (%) of 6 spiked samples at 3 levels (fat soluble colors 0.5, 5, 15 mg/kg, water soluble colors 50, 150, 300 except erythrosine 75, 225,450 mg/kg)

Colors were not spiked because not relevant to food
*** Color was not detectable in matrix*

Color	Matrix	Spike Level [mg/kg]	Mean Intra-day [%]	Mean Inter-day [%]
Allura Red	wine	150	5.0	5.9
Sunset Yellow	wine	150	3.6	6.8
Erythrosine	wine	225	7.6	12.9
Allura Red	chili	150	6.3	11.9
Sunset Yellow	chili	150	4.3	17.1
Erythrosine	chili	225	13.0	17.0
Metanil Yellow	chili	5	6.7	9.0
Rhodamine B	chili	5	9.8	11.4
Sudan I	chili	5	7.5	13.3
Sudan II	chili	5	7.0	9.0
Sudan III	chili	5	9.6	12.6
Sudan IV	chili	5	6.4	9.1
Sudan Red G	chili	5	6.4	8.3
Sudan Red B	chili	5	9.0	8.5
Sudan Red 7B	chili	5	8.0	8.4
Sudan Black B 1st	chili	5	10.8	12.0
Sudan Black B 2nd	chili	5	8.2	8.1
Allura Red	sauce	150	13.5	19.1
Sunset Yellow	sauce	150	8.8	13.2
Tartrazine	sauce	150	4.5	15.9
Erythrosine	sauce	225	13.9	14.8
Metanil Yellow	sauce	5	7.4	12.1
Rhodamine B	sauce	5	10.4	15.9
Sudan I	sauce	5	11.9	16.0
Sudan II	sauce	5	9.9	9.7
Sudan III	sauce	5	9.6	11.5
Sudan IV	sauce	5	6.8	7.5
Sudan Red G	sauce	5	9.9	13.1
Sudan Red B	sauce	5	8.9	10.1
Sudan Red 7B	sauce	5	9.2	9.7
Sudan Black B 1st	sauce	5	8.8	12.4
Sudan Black B 2nd	sauce	5	6.9	8.3

Table 3: Precision: intra- and inter-day repeatability of the LC-MS method

n=6, 1 level repeated on three days for water and fat soluble colors in matrices

Analyte	Liquid Matrix (wine)		Semi-liquid Matrix (sauce)		Solid Matrix (chili)	
	LOD [mg/kg]	LOQ [mg/kg]	LOD [mg/kg]	LOQ [mg/kg]	LOD [mg/kg]	LOQ [mg/kg]
Allura Red	47.0	140.0	5.0	15.0	13.0	38.0
Sunset Yellow	50.0	150.0	12.0	35.0	8.3	25.0
Tartrazine	**	**	40.0	120.0	**	**
Erythrosine	30.0	90.0	30.0	90.0	30.0	90.0
Metanil Yellow	*	*	0.5	1.5	0.3	0.9
Rhodamine B	*	*	0.03	0.1	0.03	0.1
Sudan I	*	*	0.5	1.5	0.4	1.3
Sudan II	*	*	0.3	0.9	0.3	0.9
Sudan III	*	*	0.2	0.6	0.2	0.6*
Sudan IV	*	*	0.1	0.3	0.1	0.3
Sudan Red G	*	*	0.5	1.5	0.4	1.2
Sudan Red B	*	*	0.5	1.5	0.5	1.5
Sudan Red 7B	*	*	0.5	1.5	0.5	1.5
Sudan Black B 1st	*	*	0.03	0.1	0.03	0.1
Sudan Black B 2nd	*	*	0.3	0.9	0.3	0.9

Table 4: Limits of detection and limits of quantification (LODs and LOQs) of the LC-MS method for different matrices

Colors were not spiked because not relevant to food
**Compound not applicable for this matrix*
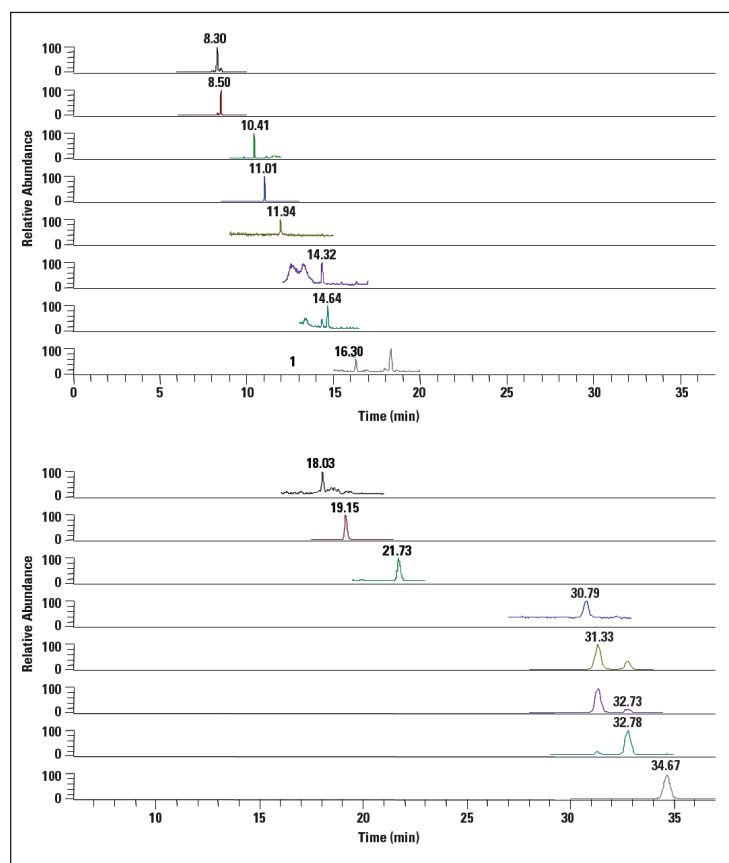
Figure 1: LC-MS chromatogram: separation and detection of all colors in sauce matrix

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