Determination of Banned Dyes in Food Source by QuEChERS and LC MS/MS Analysis

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Introduction

The red dyes Sudan I, II, III, and IV are oil soluble, azo dyes used legally in the leather and fabric industries. They are fairly inexpensive and readily available. The are not approved at any level for use in any type of food. The International Agency for Research on Cancer (IARC), part of the WHO has assessed the Sudan dyes as Group 3 genotoxic carcinogens. [1] Because their degradation products are considered to be carcinogens and teratogens, the EU and the USA do not permit the use of these colorants as food additives. However, in some countries, these dyes are still occasionally used to intensify the color of food products. Some recent additions to this group of regulated dyes include Rhodamine B, Dimethyl Yellow, Sudan Orange G, Sudan Red G, and Sudan Red 7B.

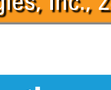
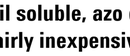
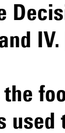
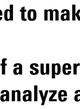
The EU issued Decision 2003/460/EC required condition of import that hot chili products be tested for Sudan I. [2] The Decision was amended in Jan 2004 (2004/92/EC) to include Sudan II,III, and IV. [3] This requirement remains in effect.

Worcestershire sauce was chosen as the food product because it is supplied to a large number of companies and is used to make over 600 different food products.

The QuEChERS methodology and the use of a superficially porous Poroshell 120 EC-C18 column were used to extract and analyze a series of dyes from food source well below the established limits (LOD) of 0.5 ppm (500 ppb).

Experimental

Table 1. Illegal Dyes and Structure Used in QuEChERS Extraction

Compound	CAS	Structure
Rhodamine B	81-88-9	
Sudan Orange G	2051-85-6	
Dimethyl Yellow	60-11-7	
Sudan Red G	1229-55-6	
Sudan I	842-07-9	
Sudan II	3118-97-6	
Sudan III	85-86-9	
Sudan IV	85-83-6	
Sudan Red 7B (IS)	6368-72-5	

Experimental

1) A series of extractions were evaluated: Original, AOAC and EN buffered salts

2) Dispersive SPE containing PSA and dispersive SPE containing both PSA and C18-EC were evaluated

3) It was determined that the AOAC salts and dispersive SPE containing PSA and $MgSO_4$ offered the best recovery.

4) The Worcestershire sample was used without a dilution

5) Illegal dye standards are made fresh daily in DMSO (1 mg/mL) and then diluted with ACN to appropriate concentrations for pre- and post spiked samples

6) The LC gradient started at 70 % ACN (0.1%) FA therefore dilution of the extract after dispersive-SPE was not necessary offering direct analysis

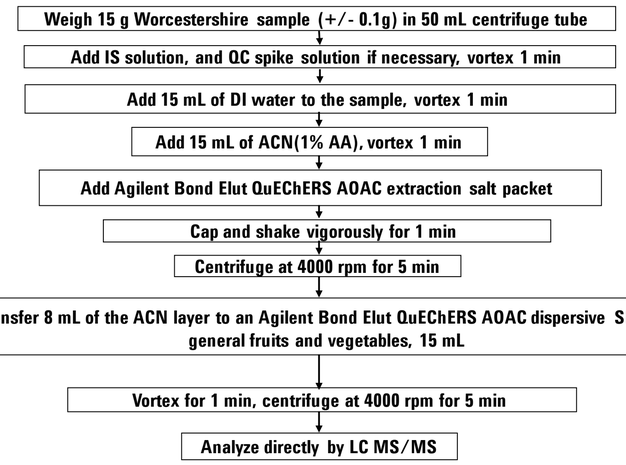


Figure 1. General Overview of QuEChERS Extraction Procedure

Results

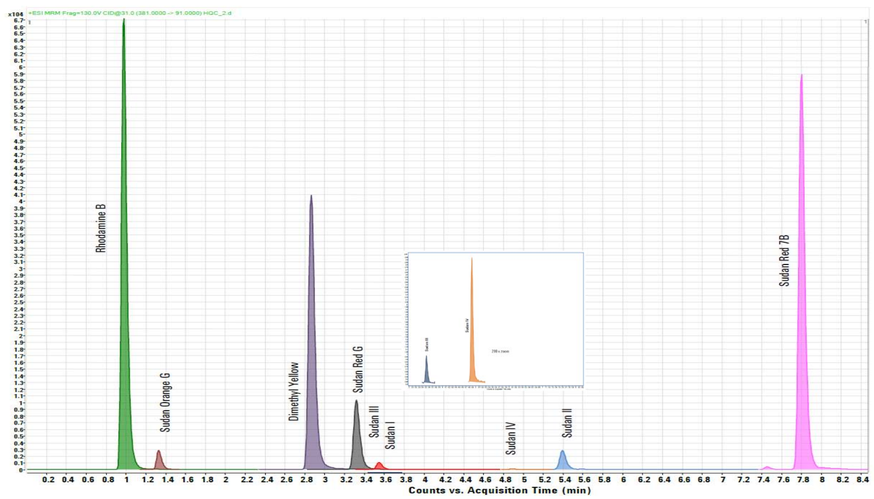


Figure 2. MRM chromatograms of 50 ng/mL pre-spiked sample processed by QuEChERS method. Peaks are identified on the chromatogram with zoomed insert for Sudan III and IV; Sudan Red 7B (IS).

Results and Discussion

Table 2. Recovery and Reproducibility of Illegal Drugs in Food Product with QuEChERS

Analytes	1 ng/g Spiked Sample		10 ng/g Spiked Sample		50 ng/g Spiked Sample	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Rhodamine B	84.9%	1.2%	92.1%	3.3%	99.7%	3.5%
Sudan Orange G	84.7%	9.0%	101%	1.5%	105%	1.3%
Dimethyl Yellow	75.7%	6.5%	98%	3.3%	106%	1.8%
Sudan Red G	106%	2.8%	100%	2.0%	98.3%	2.7%
Sudan I	64%	21.5%	97.8%	8.0%	101%	0.4%
Sudan II	105%	6.8%	99.3%	4.0%	102%	2.8%
Sudan III	121%	26%	123%	6.9%	133%	20%
Sudan IV	115%	4.3%	99.8%	2.9%	113%	16%

Discussion

The QuEChERS methodology is well suited for the extraction of illegal dyes from food products because it offers a direct analysis by LC MS/MS without evaporation/reconstituting or dilution.

The recovery and reproducibility were evaluated by spiked dye standards in Worcestershire sauce extracts at levels of 0.1, 1, 5, 10, 50, and 100 ng/mL (ppb). The QC samples were quantitated against the matrix spiked calibration curve.

The recovery and reproducibility (shown as RSD) data are in Table 2. It can be seen from the results that the 8 dyes give good recoveries and precision. The linearity of response over three orders of magnitude was observed ($r^2 > 0.99$) for the majority of the dyes tested.

Incorporating the Poroshell 120 EC-C18 is an excellent column choice for the analysis of samples where "just enough" sample preparation in employed. In part because it has standard 2 µm frits and is more forgiving for complex samples relative to sub-2 µm columns. The efficient mass transfer equates to faster analysis time, and higher throughput with optimum resolution (Figure 2).

Conclusions

Agilent Bond Elut AOAC buffered extraction kit and AOAC dispersive SPE kit for general fruits and vegetables provided a simple, fast and effective method for the extraction of illegal dyes from Worcestershire sauce. The recovery and reproducibility, based on matrix-spiked standards, were acceptable for the illegal dyes determination in Worcestershire sauce. The impurities and matrix effects from the food product were minimal although a peak was observed but did not interfere with quantitation of any target compound. The LODs of the illegal dyes were significantly lower than the general EU limits of 0.5-1 ppm.

1. IARC Summaries and Evaluations; Sudan I; International Agency for Research on Cancer; 1975.
2. 2003/460/EC: Commission Decision of 20 June 2003 on emergency measures regarding hot chili and hot chili products (notified under document number C[2003] 1970) OJ L154/114 (21.6.2003).
3. 2004/92/EC: Commission Decision of 21 January 2004 on emergency measures regarding chili and chili products (notified under document number C[2004] 68) OJ L27/52 (30.1.2004).