

Colorful, but hazardous:

Fast LC/MS determination of Sudan Red I-IV,

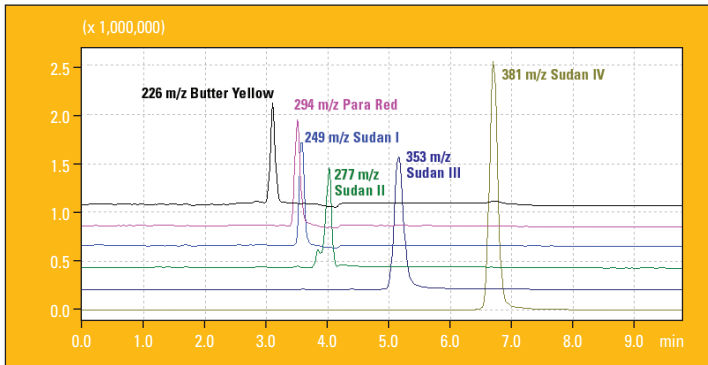


Figure 1: Mass traces of the separation of Butter Yellow, Para Red and Sudan Red I-IV on a Pathfinder® AS Silica 100 column (3.5 µm, 2.1 x 50 mm) in the positive mode

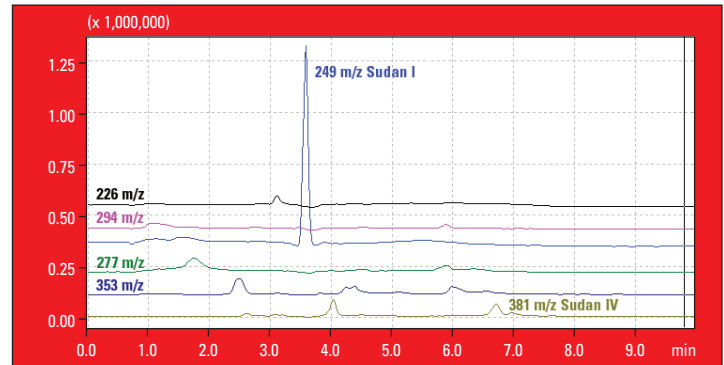


Figure 2: Chromatogram of an extract of a chicken spice mixture containing the prohibited Sudan Red I and IV, dyes, measured in the positive ionization mode

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In May 2003, the European early warning system issued a warning from France on the detection of Sudan Red dye in Indian chilli powder. During further investigations, the dyes Sudan Red II-IV had also been detected in various foods such as meat products, colored pastas, paprika powder, spice mixtures, tomato sauces as well as palm oils.

Sudan dyes are synthetic, fat-soluble red and orange-colored azo dyes. They are used as colorings for numerous household products such as floor waxes or shoe polish. However, the use of azo dyes is no longer permitted in foods, since the European Union has banned the use of Sudan-based dyes as food colorings.

In addition to the Sudan dyes, the 'Chemisches Untersuchungsamt Bergisches Land' in Wuppertal, Germany has detect-

ed Butter Yellow (dimethyl yellow) in curry and nitroaniline red (Para Red) in paprika powder. These dyes are also prohibited as food colorings.

Since January 2004, chillies and chilli products are specifically tested for the presence of Sudan Red I-IV upon import. These spices and foods may be imported into the EU only when the appropriate analysis certificate is present. In addition, food control authorities carry out random checks on products on the market. In the first half of 2004, 125 violations had already been detected.

LCMS for selectivity and sensitivity

In chilli powders and spice mixtures, the prohibited dyes were usually detected in relatively high concentrations. When these spices were used in the production of meat products, however, the concentration of the prohibited dyes decreased down to several milligrams per kilogram in the end product, for instance in spicy sausages. Since a positive test

result for these dyes requires the complete shipments to be destroyed, the applied testing procedures require analytical methods with the highest sensitivity and reliability.

The usual detection methods for Sudan dyes use liquid chromatographic separation methods, usually coupled to diode-array detection. In order to increase the sensitivity as well as the selectivity, mass spectrometry is the preferred detection mode. The above-mentioned dyes can be ionized using common LC/MS ionization modes such as atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). In the method proposed by the 'Chemisches Untersuchungsamt' of Bielefeld, Germany, the analytes of interest are separated using semi-micro HPLC and are detected via APCI and a single-quadrupole mass spectrometer (Shimadzu LCMS-2010).

Fast detection method

The sample preparation procedure is very straightforward.

forbidden dyes

Butter Yellow and Para Red in foods

One gram of sample is extracted with acetonitrile for 30 min. An aliquot is withdrawn and filtered. The sample is subsequently injected directly into the LC/MS system. Depending on the dye concentration, a dilution step may be necessary.

The Sudan Red dyes I-IV are extremely lipophilic azo dyes which are strongly retained on reversed-phase columns. Short analysis times can be attained using short chromatographic columns. For the proposed application, a 50 x 2.1 mm column was selected. A water/methanol gradient (65 - 100 %, 200 μ L/min) was used to elute the six dyes from a Shimadzu Pathfinder® AS column within seven minutes. Figure 1 shows the chromatogram of mass traces of a separation of the four Sudan Red dyes as well as Butter Yellow and Para Red.

The extrapolated detection limits in a matrix-adjusted calibration setting are, for Sudan Red I-IV and Butter Yellow between 5 and 20 pg total (RMS noise of S/N 5:1) and 20 pg total for Para Red. Using this method with 10 μ L injection volumes, concentrations into the lower μ g/L range can be detected.

As the concentrations of Sudan Red detected in spices were between 2 and 4 g/kg and in spice mixtures between 40 and 150 mg/kg, the attained detection limits are at present sufficient. Experiments with spiked matrix solutions showed no significant ion suppression for all analytes. Spiked matrix samples were linear in the tested range of 0.1 mg/L up to 2.0 mg/L.

Figure 2 presents the chromatogram of mass traces of an extract of a positively tested chicken

spice mixture (1:10 dilution, 1 μ L injection volume). In this extract, 132 mg/kg Sudan Red and 5 mg/kg Sudan Red IV were detected.

Apart from Butter Yellow, the dyes can also be ionized in the negative mode. Negative ionization can, for instance, be used for the verification of inconclusive analysis results obtained during the positive ionization mode.

Summary

The single quadrupole LC/MS system is a sensitive and selective method and is especially suitable for the determination of Sudan Red I-IV, Butter Yellow and Para Red. The robustness of the instrument allows a simple sample preparation procedure. With a 50 mm long separation column, the dye mixture can be analyzed within seven minutes. The detec-

tion limits for the azo dyes using this method are within a range of 5 - 20 μ g/kg food sample, and more gain in sensitivity can be attained through variation of the sample preparation procedure or increasing the injection volume.

LC/MS conditions	
Separation column: Pathfinder® AS 100; 50 x 2.1 mm; 3.5 μ m Shimadzu/Shant Laboratories	
Eluents: A = Water (Milli-Q®), B = methanol (HPLC-grade, Roth)	
Gradient:	
Initial concentration:	65 % B
0 - 1 min	linear up to 95 % B
1 - 3 min	linear up to 100 % B
3 - 10 min	isocratic 100 % B
5 min	equilibrate at 65 % B
Flow rate:	200 μ L/min
Temperature:	55 °C
MS-parameter positive ionization	
Mode:	Atmospheric Pressure Chemical Ionization (APCI) positive
Scan range:	100 - 500
SIM masses:	Sudan I 249 m/z [MH] ⁺
	Sudan II 277 m/z [MH] ⁺
	Sudan III 353 m/z [MH] ⁺
	Sudan IV 381 m/z [MH] ⁺
	Butter Yellow 226 m/z [MH] ⁺
	Para Red 294 m/z [MH] ⁺

