SHIMADZU APPLICATION NEWS

LAAN-A-LM-E011

● LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

No.C54

Analysis of Sudan Dyes and Para Red using LC-MS

Sudan dyes are red or orange synthetic pigments used to color various dyeing oils and waxes, etc. Sudan dyes are not permitted in food in many countries, including Japan, the EU and the United States, due to concerns that they may be carcinogenic. However, there are some countries where these dyes are still being used for coloring of such spices as chili powders and paprika. Since Sudan Ⅰ was first detected in cayenne products imported into the EU in May 2003, there have been many reported cases of its detection, and this has led to strengthened inspection of such products when they are imported into Japan.

The Ministry of Health, Labour and Welfare Administration of Food Safety Publication No. 0501008, “Test Methods for Sudan Dyes and Para Red in Foods” (hereafter referred to as “official methods”), dated May 1, 2006, specifies the use of HPLC as the quantitation method, and LC-MS as one of the confirmation test methods.

This Application News introduces an example of simultaneous quantitative analysis of Sudan dyes and Para Red using LC-MS.

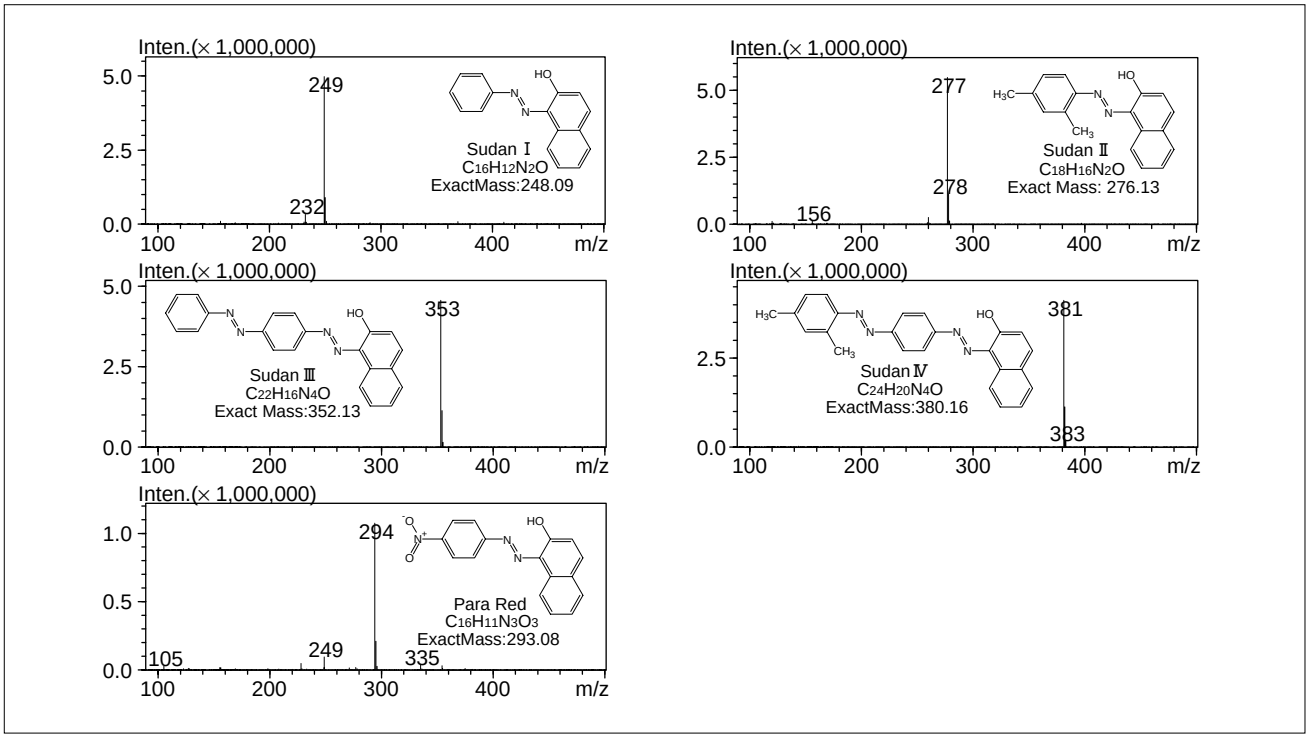

Fig.1 Positive ESI Mass Spectra of Sudan Dyes and Para Red

Among the official methods, the ionization mode specified for use in LC-MS is positive ion electrospray ionization (ESI-positive). A scan measurement was performed on a standard sample to check the spectra, and a SIM measurement to check the linearity. Fig.1 shows the structural formulas and mass spectra for Sudan Ⅰ – Ⅳ and Para Red. In all cases, the protonated molecule ($[M+H]^+$) was detected as the base peak. Fig.2 shows the calibration curves with concentrations from 5 to 1000 ng/mL (n=5). Excellent linearity was obtained in all cases, with correlation coefficients of 0.998 or greater. Moreover, each compound was adequately confirmed at 1/10 the concentration of the detection limit of 0.5 μg/g (final solution concentration of 50 ng/mL following pretreatment) specified in the official method. The official method also requires the 5 μg/g spike recovery rate for quality (precision) control. We conducted spike recovery tests for accuracy verification of Sudan Ⅰ – Ⅳ and Para Red in commercial chili powder according to the official method. SIM with good sensitivity and precision was used for the measurement. For pretreatment, each 25 μg of standard preparations was added to 5 g of chili powder, as specified in the official method. The concentration of the final solution after pretreatment is 500 ng/mL when assuming 100 % recovery. Fig.3 shows the SIM chromatograms obtained for Sudan Ⅰ – Ⅳ and Para Red added to commercial chili powder. The black-colored chromatograms were obtained from measurement of chili powder spiked with the standard preparation, the red-colored chromatograms are of the unspiked chili powder, and the blue-colored chromatograms are of the standard sample. Good values were obtained in all cases, with the recovery rates from 97.9 % to 108.0 %, and recovery coefficients of variation (n=3) from 0.81 to 2.00 %.

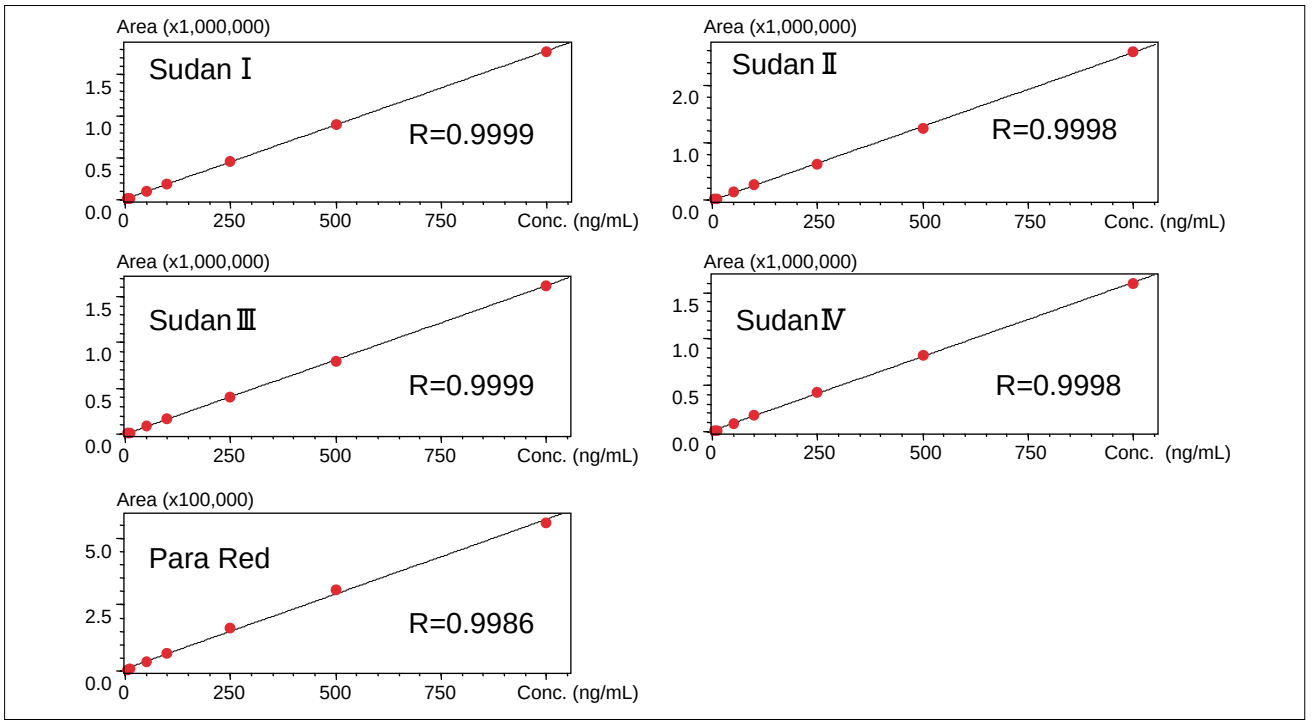

Fig.2 Calibration Curves of Sudan Dyes and Para Red

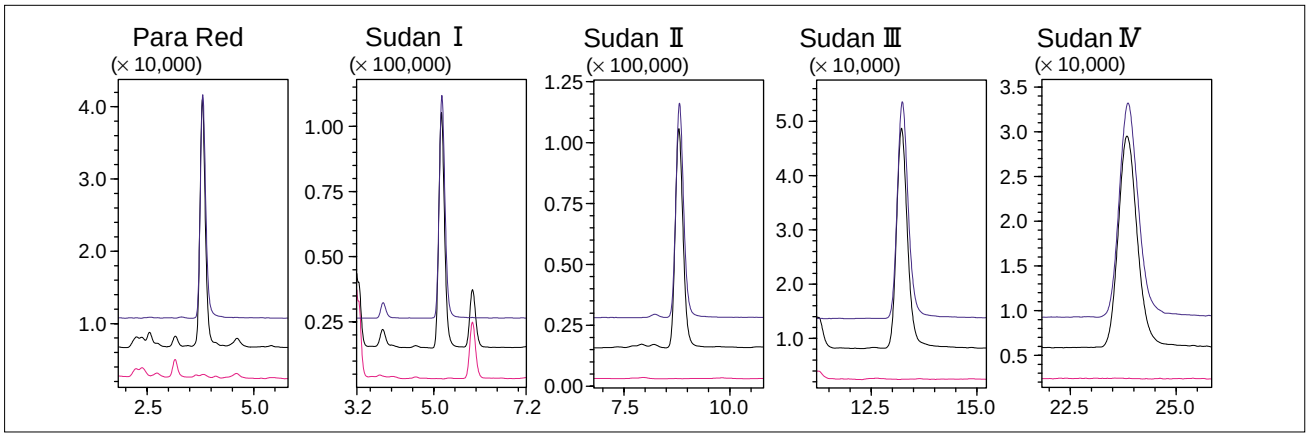

Fig.3 SIM Chromatograms of Chili Powder Extract with 5 μg/mL Sudan Dyes and Para Red (Black), Chili Powder Extract (Red), and Sudan Dyes and Para Red (Blue) at 500 ng/mL each

Table 1 Analytical Conditions for LC-MS

Column	: Shim-pack FC-ODS (2.0 mmI.D. × 150 mm)		
Mobile phase	: water containing 0.1 % formic acid/acetonitrile = 15/85		
Flow rate	: 0.2 mL/min		
Injection volume	: 1 μL	Column temperature	: 40 ˚C
Probe voltage	: 4.5 kV (ESI-Positive mode)		
CDL temperature	: 250 ˚C	BH temperature	: 200 ˚C
Nebulizing gas flow	: 1.5 L/min	Drying gas pressure	: 0.10 MPa
CDL voltage	: using default value		
Q-array DC voltage	: using default value	Q-array RF voltage	: using default value
Scan range	: m/z 90-500	SIM monitoring ions	:m/z 294,249,277,353,381

NOTES:
✻This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

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