



Rapid Identification of Illegal Dyes in Paprika Oil using HPLC with ELSD

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Introduction

The Varian evaporative light scattering detector is universal and is not dependent on the optical properties of the compound. Consequently, ELSD offers a uniform response for compounds like sudan dyes that are UV active but with differing extinction coefficients.

Sudan I, II, III and IV are oil-soluble azo dyestuffs, which are legally used in the fabric and leather industry. They have been classified as category 3 carcinogens by the International Agency for Research on Cancer¹, and, consequently, they are not approved for use in food products at any level. There have been many instances of food adulteration using sudan dyes², the most recent being in the UK, where the presence of Sudan I was detected in Worcestershire sauce, which in turn was an ingredient of many foodstuffs. While the EU Commission Decision does not specify the method of analysis for Sudan I-IV, the use of validated methods by official food control laboratories is an important requirement of the EU³. A number of HPLC methods, some resulting from collaborative trials⁴, have been presented for the detection of illegal dyes in foodstuffs, typically using diode-array UV and mass spectrometry (MS). The current EU limit of detection (LOD) for Sudan I and other similar dyes is 0.5–1.0 mg/kg, which is achievable using UV detection. However, false positives can arise from carotenoids present in the sample, which have a similar absorbance range to sudan dyes, so the choice of UV wavelength used for quantification differs between HPLC method. To maximize the sensitivity of HPLC/UV methods for these dyes, UV detection needs to be performed at four different wavelengths, namely 476 nm, 493 nm, 512 nm and 357 nm for Sudan I, II, III and IV, respectively⁵. For single wavelength detection, 480 nm is often used⁴, but the response of Sudan III and IV at this wavelength is compromised.

Instrumentation

Column: C18 5 μ m, 150 x 2.1 mm

Detection: Varian ELSD (neb=30 °C, evap=50 °C, gas=1.6 SLM)

Materials and Reagents

Eluent A: 0.1 % Acetic acid in water

Eluent B: 0.1 % Acetic acid in ACN

Sample Preparation

A sample of paprika oil was dissolved in ACN/water to give a concentration of 50 μ g/mL.

Conditions

Flow Rate: 0.233 mL/min

Injection Volume: 20 μ L

Gradient: 70–95 % B in 10 min; hold 10 min

Results and Discussion

Sudan I and II were identified in the paprika oil, as shown in Figure 1. The components of the paprika oil were sensitive to temperature and maximum sensitivity was obtained at an ELSD temperature of 25 °C. Paprika oil also contains other dyes, such as bixin, Sudan Red 7B and Sudan Orange B but the identification of these species, as well as Sudan III and IV was not possible, as individual standards were not available.

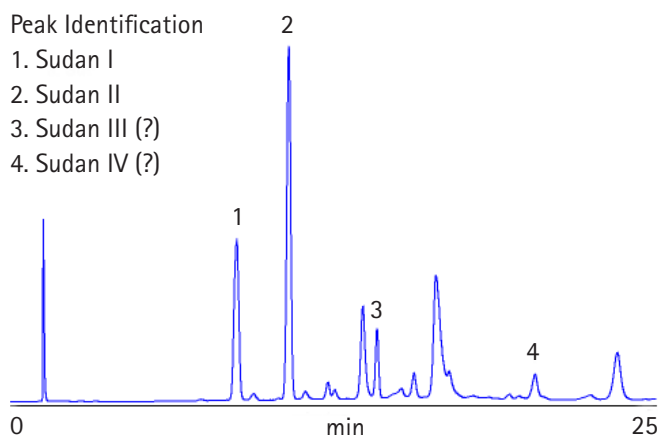


Figure 1. Clear peaks indicating illegal sudan dyes in paprika oil with the Varian ELSD at 30 °C.

Conclusion

A simple reversed phase HPLC separation of Sudan I-IV was achieved using evaporative light scattering detection. The combination of the Varian ELSD and HPLC provides a fast and simple method for determining sudan dyes in foodstuffs.

The Varian ELSD surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELSD's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian ELSD provides superior performance compared to competitors' detectors for the analysis of semi-volatile compounds.

References

- ¹Sudan Red and Related Dyes White Paper: <http://www.astaspice.org/pubs/SudanWhitePaper.pdf>
- ²<http://www.food.gov.uk/foodindustry/guidancenotes/foodguid/sudanguidance>
- ³Implementation of Commission Decision 2004/92/EC of 21 January 2004: http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_027/l_02720040130en00520054.pdf
- ⁴Collaborative Trial 145 of a method for the detection and determination of Sudan I in chilli products by HPLC. <http://www.food.gov.uk/multimedia/pdfs/sudan145a.pdf>
- 5Kowalski, J (2005) Simple HPLC Analysis for Sudan Dyes: Monitor Sudan I, II, III and IV in a Single, Isocratic Analysis. http://www.restek.com/aoi_fff_A001.asp

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