

Easy Isocratic HPLC Determination of Sudan Dyes

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In May 2003, France issued a warning against chili powders from India that have been contaminated with the Sudan dye Sudan I. Sudan dyes are azo dyes.¹ These can be metabolized to primary amines, which are suspected carcinogenic compounds. Therefore, in the European Union it is not allowed to use these dyes for coloring food or foodstuff. Since June 2003, importers or food manufacturers must include a certificate of analysis showing the absence of these compounds (Sudan I - IV)² in all shipments of chili powder and products containing chili powder. The EU national food administrations are forced to control this on an ongoing basis. As evidence of this ongoing concern, in February 2005 the largest recall of processed food (fish sauce, worchester sauce, noodle soup, and pizza) occurred in the United Kingdom due to chili powder contaminated with Sudan I.

Introduction

The goal of this work was the development of an easy and isocratic HPLC method for the determination of Sudan dyes (Sudan I-IV and Sudan Red B). The separation of the two isomers Sudan IV and Sudan Red B is especially challenging for the chromatographer as it is not possible to distinguish between these two compounds in LC/MS if they are co-eluting. Conventional C18 phases do not have the right selectivity to solve this task in an easy manner. Thus we decided to use a unique, polar-embedded phenyl phase (Synergi™ Polar-RP®) for the method development. To enhance pi-pi interactions, methanol was chosen as the organic modifier.

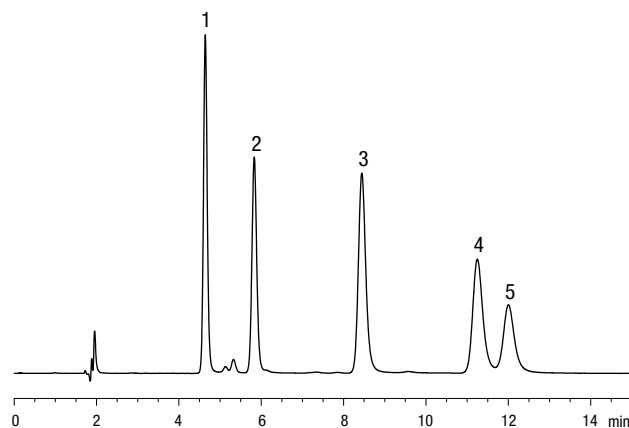
Instrumentation/Equipment

Analysis was performed using an HP 1100 LC system, equipped with a quaternary pump, in-line degasser, diode-array detector and autosampler (Agilent Technologies, Palo Alto, CA, USA). The HPLC column used for the analyses was a Synergi™ Polar-RP® 150 x 4.6 mm (Phenomenex, Torrance, CA, USA). All solvents were HPLC grade and were obtained from Burdick and Jackson (Muskegon MI, USA). All Sudan dyes were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Experimental

Chromatographic conditions: A tertiary mobile phase of water/acetonitrile/methanol (15:20:65) was used, with a flow rate of 1 mL/min. The column temperature was 30°C. UV-Visible detection was recorded at a wavelength of 480 nm.

Figure 1. Separation of Sudan Dyes



App ID 15890	Sudan Dyes using Synergi™ 4µm Polar-RP®
Column:	Synergi 4µm Polar-RP 80Å
Dimensions:	150 x 4.6mm ID
Order No.:	00F-4336-E0-TN
Elution Type:	Isocratic
Mobile Phase:	Water/Acetonitrile/Methanol (15:20:65)
Flow Rate:	1 mL/min
Col. Temp:	30°C
Detection:	UV-VIS @ 480 nm
Analytes:	1. Sudan I 2. Sudan II 3. Sudan III 4. Sudan IV 5. Sudan Red B

Results

The choice of a polar-embedded phenyl phase (Synergi™ Polar-RP®) and the use of methanol in the mobile phase resulted in a good separation of the two isomeric Sudan dyes (Sudan Red B and Sudan IV). The method enables the quantitation of Sudan I-IV and Sudan Red B in one run within 15 minutes.

References

1. Commission Decision 20 June 2003. Official Journal of the European Union L154/114.
2. Annual Report 2004. Rapid Alert System for Food and Feed. European Commission Health & Consumer Protection Directorate General.

ORDERING INFORMATION

Order Number	Description
00F-4336-E0-TN	Synergi™ 4µm Polar-RP® 150 x 4.6mm

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