“Fast peaks” in chromatograms of Sudan dyes

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Abstract

The analysis of Sudan dyes has gained considerable attention during last years. In several publications multiple peaks in chromatograms of Sudan III and Sudan IV have been detected. It is demonstrated in this work that if the sample is kept in darkness before analysis, only one chromatographic peak appears for Sudan III or Sudan IV. In normal lighting conditions two peaks are observed. This light-induced effect can lead to under- or overestimation of the Sudan III and Sudan IV content by 10% and false-positive detection of Sudan I and Sudan II, if proper care is not taken.

Appearance of the second peak in chromatograms of Sudan III and Sudan IV is attributed to photochromic E–Z isomerization of these dyes.

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In our experiments we also observed two peaks with vastly different retention times (7.4 and 18.4 min; see below) in the chromatograms of a Sudan III standard substance solution. The peaks belonged to compounds with identical molecular masses and fragmentation spectra but different UV/Vis spectra and apparently very different polarities. In this paper, we provide evidence that the two peaks are due to photochromism of Sudan III and IV. The effects on analytical results are discussed.

2. Experimental

2.1. Reagents

2.1.1. Standards

Standards of Sudan I (1-(phenylazo)-2-naphthalenol CAS Registry Number (CAS No.): 842-07-9; Colour Index (CI) 12055) purity 97% (Sigma–Aldrich, Sigma–Aldrich GmbH, Steinheim, Germany); Sudan II (1-[(2,4-dimethylphenyl)azo]-2-naphthalenol CAS No. 3118-97-6; CI 12140) purity 90% (Aldrich, Sigma–Aldrich GmbH), Sudan III (1-(4-phenylazophenylazo)-2-naphtalenol; CAS No. 85-86-9; CI 26100) purity 95% (Sigma, Sigma–Aldrich GmbH) and Sudan IV (1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo]-2-naphtalenol; CAS No. 85-83-6; CI 26105) purity 80% (Aldrich, Sigma–Aldrich GmbH).

2.1.2. Solvents and solutions

Methanol (HPLC-grade purity) was obtained from J. T. Baker (Mallinckrodt Baker, B.V., Deventer, The Netherlands); ace- tonitrile Chempure (Curtin Matheson Scientific Inc., Houston, Texas, USA) GC pesticide residue analysis grade; and acetone (HPLC-grade purity) from Rathburn (Rathburn Chemicals Ltd, Walkerburn, Scotland, UK); formic acid from Riedel-de Ha¨en (Sigma–Aldrich GmbH) (purity > 98%) and ammonium acetate from Fluka (Sigma–Aldrich GmbH) (purity > 99%). The buffer solution was 1 mM ammonium acetate + 0.1% formic acid in deionized water.

2.2. Apparatus and conditions

Agilent (Agilent Technologies Deutschland GmbH, B¨oblingen, Germany) LC–MS system was used, consisting of 1100 series LC system with DAD (detection at two wavelengths $\lambda_1 = 480$ and $\lambda_2 = 540$ nm, bandwidth 10 nm, reference 650 nm, bandwidth 100 nm; also full spectrum was registered between 190 and 900 nm), followed by ESI source and MSD Trap XCT (ion trap mass spectrometric detector).

Clear (part number 5183-4429) and amber (part number 5181-3375) 2 ml vials by Agilent (Agilent Technologies Deutschland GmbH) were used to assess the usefulness of amber vials.

Fig. 1. Chemical structures of Sudan dyes I–IV.
Fig. 4. The UV/Vis chromatogram of Sudan I–IV standard solution in methanol (2 mg/l).

Fig. 5. The EIC traces of Sudan I–IV standard solution in methanol (2 mg/l).
Chromatographic separation was performed on an Agilent (Agilent Technologies Deutschland GmbH) Zorbax Eclipse XDB-C18 analytical column (250 mm in length, ID 4.6 mm and 5 μm particle size) with an Agilent (Agilent Technologies Deutschland GmbH) precolumn Eclipse XDB-C18 (12.5 mm in length, ID 4.6 and 5 μm particle size). Gradient elution with acetonitrile (B) and buffer solution (A) at 0.8 ml/min was applied: 10 min isocratic at 82% (B), followed by 10 min linear gradient from 82% to 100% of B, and 13 min isocratic at 100% (B). For MS and MS2 confirmation, four suitable time windows were set in positive polarity. For all compounds automatic parameter optimization was carried out including fragmentation. Time windows, fragmentation potentials and m/z ratios for all components are given in Appendix A Table 1.

In some of the double peak experiments of Sudan III and Sudan IV Agilent (Agilent Technologies Deutschland GmbH) precolumn (Eclipse XDB-C18, 12.5 mm in length, ID 4.6 and 5 μm particle size) and isocratic elution was used with acetonitrile/buffer (90/10) as mobile phase. Separations carried out at these conditions will be referred to as "rapid separation" in the following text.

3. Results and discussion

3.1. Nature of the "fast peaks"

When a standard solution mixture of Sudan I–IV was analysed, then all in all six peaks were present in the UV/Vis chromatogram (see Fig. 4). (The peak at 10.2 min appeared to be an unidentified impurity and will not be considered here.) In the MS extracted ion chromatogram (EIC) for Sudan I (249 m/z) and II (277 m/z) only one peak appeared, whereas for Sudan III (353 m/z) and IV (381 m/z) in MS and MS2, two peaks appeared, and the mass spectra of those peaks were similar (for MS EIC traces see Fig. 5, for MS2 spectra see Fig. 6).

Presented in Figs. 4 and 5 are the UV/Vis chromatograms of a Sudan I–IV standards and extracted ion chromatograms of their quasimolecular ions [M + H]+. In the case of Sudan III and IV, one can see an additional peak ("fast peak") in the EIC trace and also the corresponding peaks in the UV/Vis chromatogram. Comparison of fragmentation mass spectra (MS2) reveals that the corresponding "fast" and "slow" peak give identical fragmentation spectra (Fig. 6). However, the UV/Vis spectra are different as demonstrated by Fig. 7. The chromatographic behaviour of Sudan dyes I–IV has been summarized in Appendix A Table 2.

In the UV-chromatogram at 480 nm the fast peak (see Fig. 4) accounts for 4.3% and 3.2% of the area of slow peak for Sudan III and for Sudan IV, respectively. In MS the percentage is 19.2 and 21.5, respectively. As the manufacturer of standard substances of Sudan III and IV guarantees only moderate purity for those dyes – 95% and 80%, respectively – it would be logical to attribute the "fast" peaks to synthesis by-products. For example, positional isomers could explain all the observations – difference in capacity factors, similar mass spectra and different UV/Vis spectra.

Fig. 6. Average MS2 spectra of Sudan III and IV "slow" and "fast" peaks.
An attempt was made to quantify the principal component of Sudan III standard substance (as Sudan IV is seen to behave similarly; further experiments were carried out using only Sudan III). The “slow peak” of Sudan III was preparatively separated on the analytical C18 column. The obtained solution was reanalysed approximately 25 min after purification. Surprisingly, the fast peak, although smaller than it was initially, had reappeared! Two more injections of the same solution, after 1 and 2 h, revealed that the “fast peak” regained its initial relative intensity (compared to “slow peak”) in less than 1 h. Such behaviour can only be explained by some kind of slow equilibrium process.

The following series of experiments were carried out to assess the influence of light on the appearance of the “fast peak”. The “slow peak” of Sudan III was again preparatively separated but this time in total darkness (even the light diodes of instruments were covered with aluminium foil; the system was controlled remotely from another laboratory). The obtained extract was analysed, and no “fast peak” was detected. When the solution was gradually brought to light (lights in the laboratory were lit starting from the least intense) the analysis of the extract showed the “fast peak” emerging again and the area increasing to a certain level (see Fig. 8).

Finally, the extract of the “slow peak” was exposed to an intense light and chromatographed—the presence of the “fast peak” was confirmed. Then the solution was stored in the dark for approximately 3 h. After that the solution was chromatographically separated (in darkness) and analysed. No “fast peak” was observed.

These experiments demonstrate that the “fast peak” is an isomerization product of the “slow peak” compound. The process is induced by light and it is reversed in the absence of light. As a further proof of the concept the mobile phase flow was stopped at the moment when the chromatographically separated “fast peak” reached the photodiode array detector. The change in the UV/Vis spectrum was recorded during 7.5 min (Fig. 9), the same was also performed for the “slow peak”.

Fig. 7. UV/Vis spectrum (registered with diode array detector) of “fast” and “slow” peaks.

Fig. 8. The MS² chromatograms of the purified (in darkness) Sudan III (rapid separation conditions): (a) the sample kept in darkness; (b) the same extract after exposure to intense light.

Fig. 9. Change of the UV/Vis spectrum of Sudan III “slow” and “fast” peaks during trapping in the cell of photodiode array detector. All spectra are normalized to maximum absorbance; the spectra of “slow peak” are offset by 20 mAU for clarity.