

# SUDAN DYES ANALYSIS

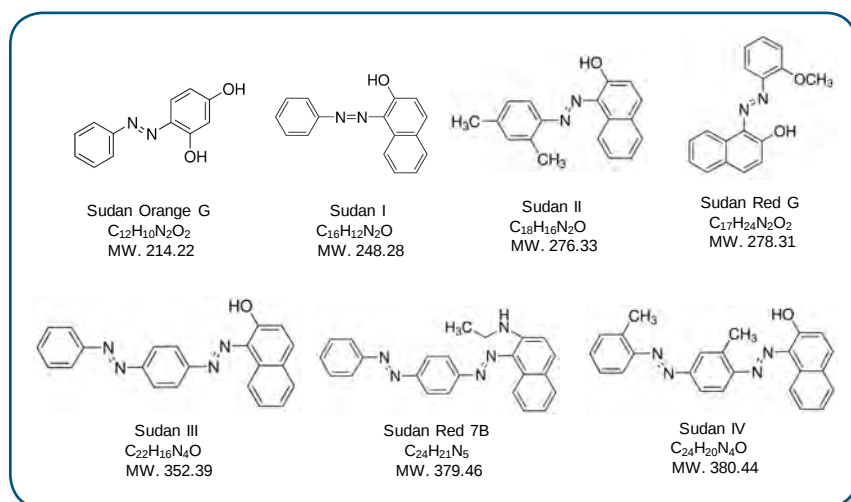
## Using Compact Mass Spectrometry with TLC Interface

Advion

### INTRODUCTION

Sudan Dyes are synthesized azo-dyes which are widely used to generate color in plastics, leather, fabrics, oil, waxes, etc. They are classified as Group 3 carcinogens by the International Agency for Research on Cancer (IARC) and are banned as food additives worldwide.<sup>1,2</sup> However, in some countries these dyes are still occasionally used in order to intensify the color in products. In this note, a TLC/CMS method using expression compact mass spectrometer (CMS) coupled with the Plate Express™ Interface (Advion, Ithaca, NY) is introduced for the detection of 7 Sudan dyes in a chili matrix. The primary advantage of TLC/CMS analysis is the ability to sample directly from the TLC plate without further sample preparation after the TLC separation is done. Molecular weight information of sample components can be determined by the mass spectra from the eluted TLC spots.

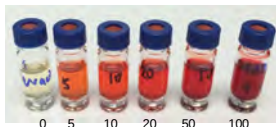
### Structures of 7 Sudan Dyes



Advion TLC/CMS system: Advion expression CMS, Plate Express, Isocratic pump.

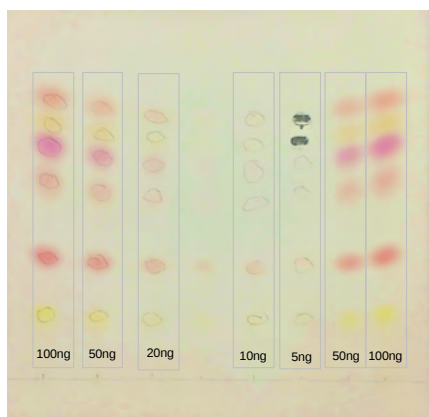
## METHOD

**Chemicals** Sudan dyes (Sudan I, Sudan II, Sudan III, Sudan IV, Sudan orange G, Sudan Red 7B and Sudan Red B) were purchased from Sigma-Aldrich (St. Louis, MO USA). Stock solutions at 1mg/mL were prepared with a solvent mixture of MeOH/CCl<sub>4</sub>, 50/50 v/v. All solvents and buffers (water, methanol, acetonitrile, formic acid and ammonium acetate) were LC/MS-grade purchased from Sigma.



**Sample preparation** Chili powder was purchased from a local food store. 2.5 g of homogenized chili powder was extracted with 25 mL of acetonitrile by stirring for 15 min, followed by filtration with a 0.4μ filter. A dilution series of concentrations of the stock solution of Sudan dyes were prepared at 0, 0.5, 5, 10, 20, 50 and 100 ng/μl by spiking them into chili powder extract.

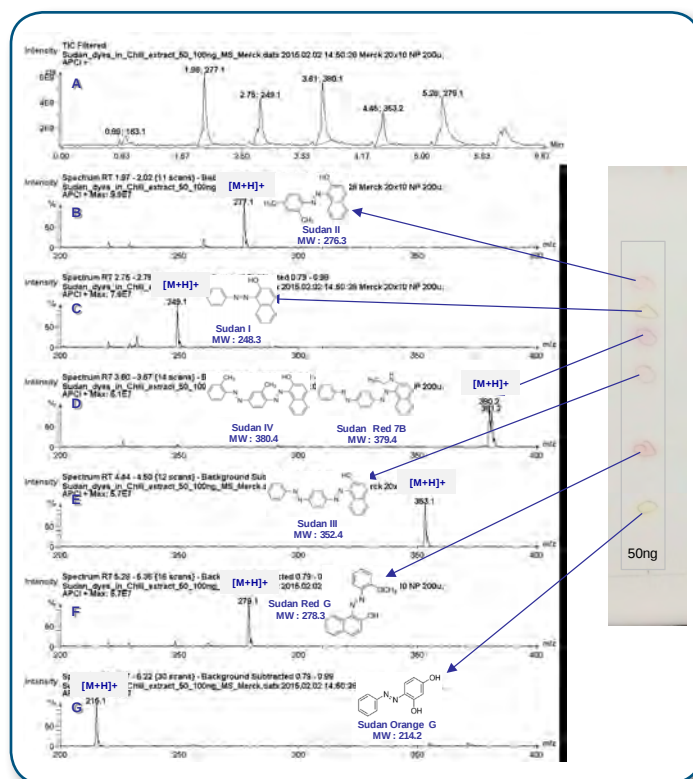
**TLC separation of Sudan Dyes** The TLC plate: Merck TLC Si60 F254. The TLC developing solvent was a mixture of ethyl acetate and hexane (15/85, v/v). For each concentration, 1 μl of sample was spotted on the Merck plate. On the developed TLC plate, 5 ng is the lowest concentration to be located under 254nm UV or visually.



**TLC/CMS Experiments** The TLC/CMS analysis of Sudan Dyes was performed on an Advion CMS single quadrupole mass spectrometer coupled with the Advion Plate Express device. The latter device is a planar surface extraction system which couples directly to the CMS ion source. The TLC plate elution solvent (0.1% formic acid in MeOH/ACN, 50/50) was delivered by an Advion LC isocratic pump with a flow rate at 250 ul/min. Positive ion APCI was used with the following source settings: Capillary Temperature(°C), 250; Capillary Voltage(V), 150; Source Voltage(V), 20; Source Temperature (°C), 350; Corona Discharge(μA), 5.

## RESULTS

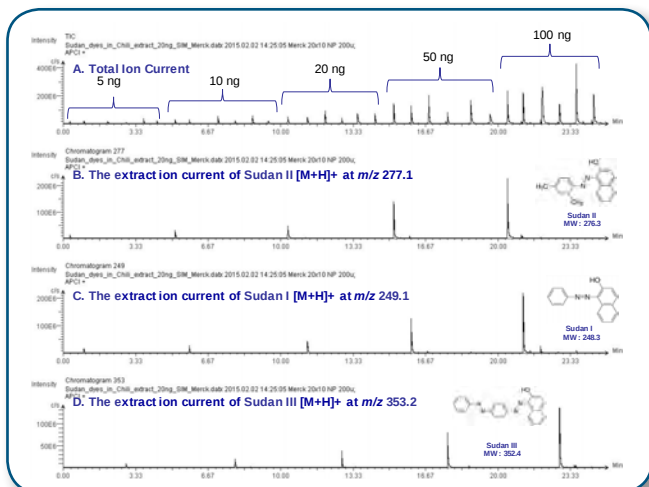
The extract of chili powder did not contain any of 7 Sudan Dyes studied in this note which is confirmed with TLC/CMS analysis (data not shown in this note). The TLC/CMS analysis of 50 ng for each dyes is shown in figure 1. Sudan IV and Sudan red 7B were not well separated by the TLC plate. This will be a challenge for a UV detector which cannot distinguish them by single wavelength. However, this issue can be addressed with TLC/CMS analysis because they each have different protonated molecules at  $m/z$  381.1 for Sudan IV and at  $m/z$  380.1 for Sudan Red 7B. All the 7 Sudan dyes can be directly analyzed and confirmed from the TLC plate separation by the Advion TLC/CMS system.



**Figure 1.** The TLC/CMS analysis of 50 ng 7 Sudan Dyes in Chili powder extract. A.) total ion current of scans from 200 to 400; B.) Mass Spectrum of Sudan II at  $m/z$  277.1; C.) Mass Spectrum of Sudan I at  $m/z$  249.1; D.) Mass Spectrum of Sudan IV and Sudan Red 7B at  $m/z$  381.1 and  $m/z$  380.1; E.) Mass Spectrum of Sudan III at  $m/z$  353.1; F.) Mass Spectrum of Sudan Red G at  $m/z$  279.1; G.) Mass Spectrum of Sudan Orange G at  $m/z$  215.1

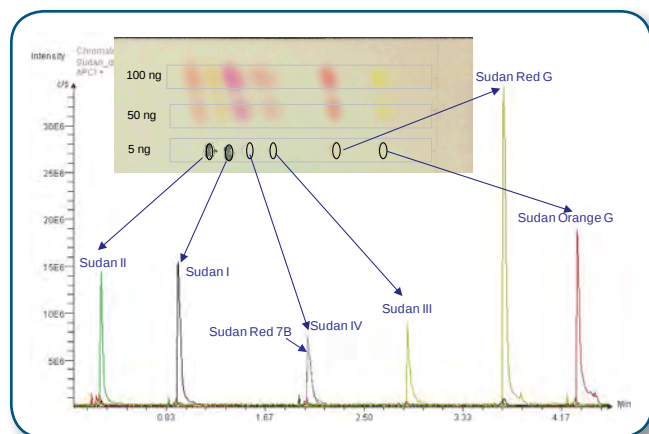
With the selected ion monitoring (SIM) mode of acquisition, the performance can be improved with lower chemical background and improved sensitivity. The different concentrations of dyes spiked into chili powder extract were (5, 10, 20, 50 and 100 ng/ul). On the developed TLC plate, the 5 ng spot was difficult to be located for all the seven dyes. Three dyes of Sudan I, II and III were used as examples for the demonstration of the TLC/CMS analysis in the SIM mode (Figure 2).

Considering the background from plate, the detection level for 7 Sudan Dyes with TLC/CMS is quite acceptable at 1.0 ng with acceptable accuracy for each of the Sudan dyes.



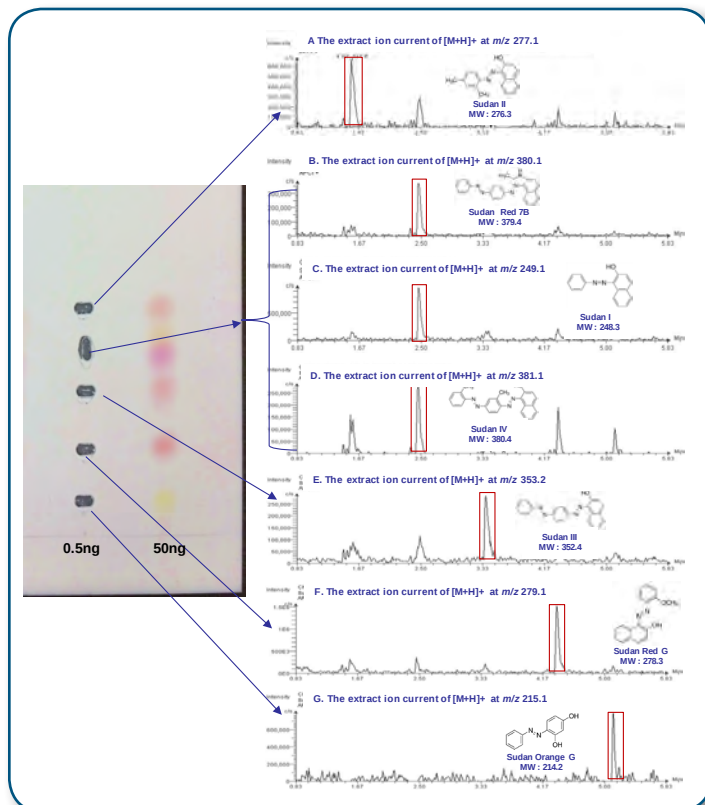
**Figure 2.** The TLC/CMS analysis of 7 Sudan Dyes in Chili powder extract at different concentrations (5, 10, 20, 50 and 100 ng). A.) total ion current of the 7 Sudan Dyes in Chili powder extract at different concentrations (5, 10, 20, 50 and 100 ng); B.) the extract ion current of the  $m/z$  277.1, Sudan II at different concentrations (5, 10, 20, 50 and 100 ng); C.) the extract ion current of the  $m/z$  249.1, Sudan I at different concentrations (5, 10, 20, 50 and 100 ng); D.) the extract ion current of the  $m/z$  353.2, Sudan III at different concentrations (5, 10, 20, 50 and 100 ng).

For the 5 ng spot, the extracted ion current (XIC) profiles of all 7 Sudan dyes are shown in figure 3. And the signal-to-noise ratios at 5 ng are 495 for Sudan orange G, 567 for Sudan I, 301 for Sudan II, 538 for Sudan Red G, 538 for Sudan III, 678 for Sudan Red 7B and 601 for Sudan IV.



**Figure 3.** The TLC/CMS (SIM scan) analysis of 7 Sudan dyes (5 ng) in Chili power extract.

An additional experiment with 0.5 ng of Sudan Dyes is shown in the Figure 4. With a reference to of 50 ng spot, all the Sudan Dyes can be detected at 0.5 ng on the plate. Even the plate has some interferences for Sudan II at  $m/z$  277.1 and Sudan IV at  $m/z$  381.1. it still can see both dyes at 0.5 ng due to their higher intensity than background.



**Figure 4.** The TLC/CMS (SIM scan) analysis of 7 Sudan dyes (0.5 ng) in Chili power extract. The extract ion currents of 7 Sudan Dyes were shown in: (A) Sudan II at  $m/z$  277.1, (B) Sudan Red 7B at  $m/z$  380.1, (C) Sudan I at  $m/z$  249.0, (D) Sudan IV at  $m/z$  381.1, (E) Sudan III at  $m/z$  353.2, (F) Sudan Red G at  $m/z$  279.1 and (G) Sudan Orange G at  $m/z$  215.1.

## SUMMARY

- Sudan Dyes in food can be screened by TLC/CMS method with the Advion expression CMS coupled with Advion Plate Express interface.
- The detection limit is < 1 ng for each of the 7 Sudan dyes.
- The Advion TLC/CMS system can directly analyze from TLC plates without further sample preparation.
- The Advion TLC/CMS system is compact for on-site analysis and is easy to use.

## LITERATURE

<sup>1</sup>Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission to review the toxicology of a number of dyes illegally present in food in the EU" The EFSA Journal (2005) 263, 1–71,

<sup>2</sup>Marie S., Vaclav M., Helena R., Petr H., and Eva F., Sudan I Is a Potential Carcinogen for Humans: Evidence for Its Metabolic Activation and Detoxication by Human Recombinant Cytochrome P450 1A1 and Liver Microsomes, CANCER RESEARCH 62, 5678–5684, October 15, 2002

**Advion**

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Advion is a leader in mass spectrometry & synthesis solutions. The expression CMS is a high performance, compact, affordable single quad mass spectrometer. Its compact size allows it to fit in space-limited labs for direct access and immediate results for chemists requiring mass confirmation, reaction monitoring, quality control and purity analysis.