# Evaluación de colorantes prohibidos en alimentos. Método automatizado de preparación de muestra.

## Aplicación 054




### RESUMEN

La familia de colorantes Sudan  Sudan I, Sudan II, Sudan III y Sudan IV todos pertenecen a la familia de colorantes  Azo. La Agencia Internacional para la Investigación del cáncer ha listado estos compuestos como potencialmente cancerígenos.  La Unión Europea EU ha dictado en la comisión 2005/402/EC su prohibición como colorantes alimentarios.

La presente aplicación evalúa al sistema robotizado Modelo Automate Q40 para la extracción automatizada QuEChERS y la determinación de colorantes Sudan en alimentos.  La Cromatografía liquida acoplada a un detector de triple cuadrupolo (LC-MS/MS) es la técnica de detección final. La cuantificación se lleva a cabo mediante curvas de calibrado de adición sobre matriz. El proceso automatizado de preparación mejora la productividad del método QuEChERS mientras los resultados de detección obtenidos se encuentran en los valores previamente establecidos.


### Introduction

Azo-dyes are synthetic dyes that are frequently used as coloring agents to enhance the color of numerous food products such as spices. Due to their break down products, they are considered carcinogens, and as a result, the European Union and the United States do not allow for the use of these synthetic dyes in food and spices.[1]

One group of Azo-dyes is known as the Sudan dyes that consist of Sudan I-IV. According to the International Agency for Research on Cancer (IARC) this group of dyes has been labeled as Group 3 genotoxic carcinogens.[2] The European Union issued a commission decision 2005/402/EC to ban such Sudan dyes as food additives.  This decision states that all hot chili products must be tested for Sudan dyes.

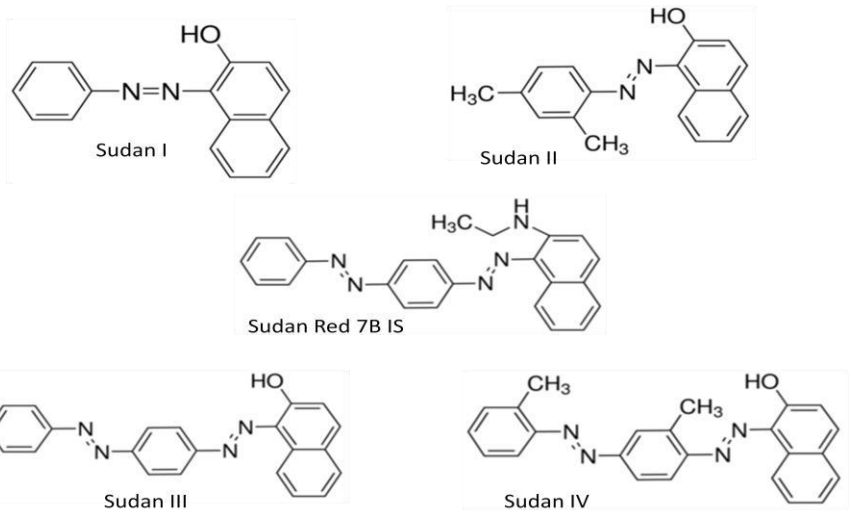
In this application note, an automated modified QuEChERS extraction procedure was utilized on the AutoMate-Q40. Liquid Chromatography coupled to a highly selective and sensitive Triple-Quadrupole Mass Spectrometer (LC-MS/MS) was employed for the detection of the Sudan dyes I-IV in chili powder.

## Experimental Instrument Conditions

### Solutions and Standards.

Sudan I, II, III and IV (Figure 1) were purchased neat from Sigma-Aldrich.[3] A stock standard solution at 1000 mg/mL of each Sudan dye was prepared in Acetonitrile. From these four stock solutions a 40 mg/mL standard solution was prepared fresh daily in Acetonitrile.

**Figure 1** Sudan dyes structures

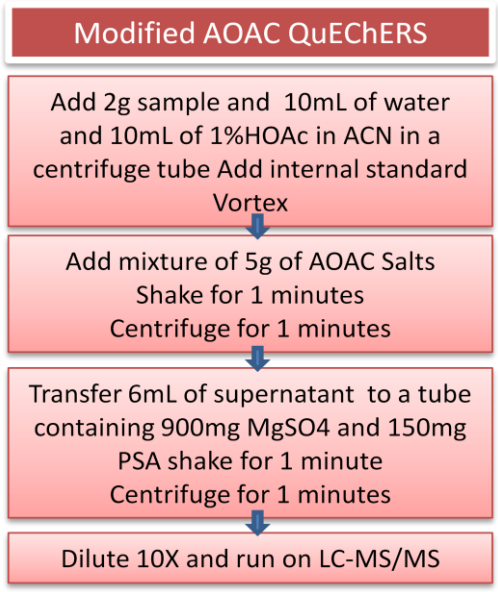



### Sample Preparation/Extraction

The sample analyzed in this application was a non contaminated organic chili powder. This sample was purchased from a local market in Ohio.

The Teledyne Tekmar AutoMate-Q40, an automated QuEChERS extraction platform, was used in conjunction with the Tek-Q AOAC consumables kit to extract Sudan dyes from chili powder (Figure 2). Two grams of sample was weighed into the 50 mL centrifuge tube and placed inside the AutMate-Q40. The AutoMate-Q40 added 10 mL of both water and 1% HOAc in Acetonitrile to the sample, which was then vortexed for 30 seconds. Once the vortex was complete the unit added the Tek-Q AOAC QuEChERS Salts. The sample was shaken for 1 minute, and then placed into the centrifuge for 1 minute. The AutoMate-Q40 removed a 6 mL aliquot from the liquid solid extraction and transferred it into the Tek-
Q dSPE tube (900 mg $MgSO_4$ and 150 mg PSA). The dSPE was shaken and centrifuged for 1 minute each. The Automate-Q40 then removed 4 mL from the dSPE tube and placed the aliquot into the final extract tube.

**Figure 2** Modified AOAC QuEChERS Extraction




## Instrumentation and Analytical Conditions

Sample analysis was conducted using a Shimadzu Nexera LC interfaced to a Sciex 4500 QTrap MS/MS. For separation of the compounds of interest, a Phenomenex Kinetex 2.6u Biphenyl 100 Å (50 x 2.1mm) column was used. Table I and Table II demonstrate the optimized LC-MS/MS analysis parameters for both the chromatographic separation and optimal analyte transitions. Figure 3 shows the scheduled MRM chromatogram spiked at 50 µL/L.

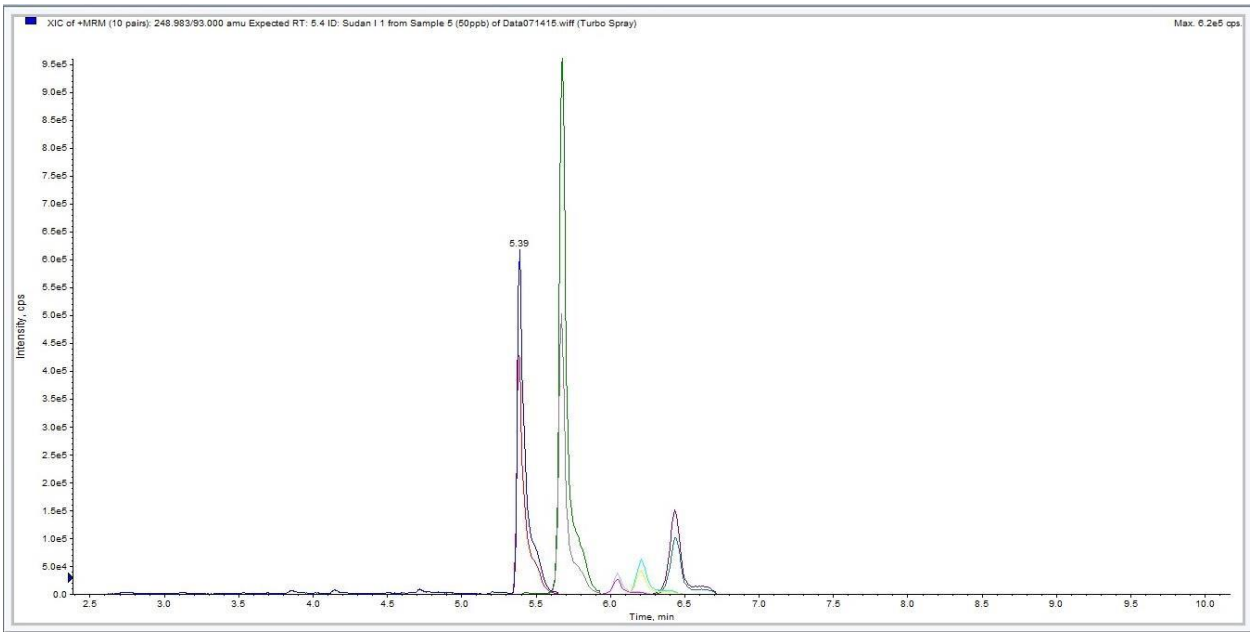
**Table I Critical LC-MS/MS SRM Transitions and Parameters for AB Sciex 4500 QTrap**

| | |
|---|---|
| Curtain Gas (CUR) | 40 |
| Ion Spray Voltage (IS) | 5500 |
| Temperature (TEM) | 450 |
| Collision Gas (CAD) | Medium |

| Analyte Transitions | | | |
|---|---|---|---|
| Compounds | RT (min) | Precursor Ion (m/z) | Quantization product Ion (m/z) |
| Sudan I | 5.39 | 248.983 | 93.000 |
| Sudan I | 5.39 | 248.983 | 155.900 |
| Sudan II | 5.67 | 277.007 | 121.000 |
| Sudan II | 5.67 | 277.007 | 106.000 |
| Sudan III | 6.06 | 353.031 | 197.000 |
| Sudan III | 6.06 | 353.031 | 128.000 |
| Sudan IV | 6.45 | 381.020 | 223.900 |
| Sudan IV | 6.45 | 381.020 | 225.000 |
| Sudan Red 7B (IS) | 6.22 | 379.968 | 183.000 |
| Sudan Red 7B (IS) | 6.22 | 379.968 | 115.000 |

| Table II Shimadzu Nexera LC P arameters | | |
|---|---|---|
| Column | Phenomenex Kinetex 2.6u Biphenyl 100 Å | |
| Dimensions | 50 X 2.00 mm | |
| Mobile Phase | A:5mm Ammonium Acetate in $H_2O$ | |
| | B:5mm Ammonium Acetate MeOH | |
| Gradient | Time | %B |
| | 0.1 | 5% |
| | 5.0 | 98% |
| | 14.0 | 98% |
| | 14.1 | 5% |
| | 15.0 | STOP |
| | | |
| Flow Rate (mL/min) | 0.400 | |
| Column Temperature (°C) | 40 | |

**Figure 3** Scheduled MRM chromatogram spiked at 50 µL/L of Sudan dyes



## Results

Automating the extraction using the AutoMate-Q40 for chili powder allows for easy, reliable and highly reproducible extractions. The AutoMate-Q40 offers labor savings, and improves the repeatability and consistency between samples.

A precision and accuracy study was performed using the AutoMateQ40. The system was able to fortify chili powder samples at 0.5, 1.0 and 2.0 µg/g. This is accomplished through the systems automated standard addition feature. All control samples used for this study showed no significant residue of Sudan dyes.

**Table III Sudan Dyes Recovery Study**

| Compound | 0.5ppm Spike | | 1.0ppm Spike | | 2.0ppm Spike | |
|---|---|---|---|---|---|---|
| | %Recovery | %RSD | %Recovery | %RSD | %Recovery | %RSD |
| Sudan I | 96.54 | 7.84 | 109.36 | 5.27 | 113.64 | 2.44 |
| Sudan II | 93.47 | 5.91 | 103.46 | 4.93 | 105.59 | 2.16 |
| Sudan III | 77.89 | 2.99 | 90.50 | 3.23 | 92.52 | 4.37 |
| Sudan IV | 68.13 | 9.42 | 76.68 | 3.11 | 79.87 | 3.31 |

The recovery and reproducibility is shown in Table II. Recovery ranged from 68 to 113%, while the reproducibility ranged from 2 to 9%. The chili powder sample is a complex matrix that will lead to suppression of the analytes of interest. An internal standard can be used (Sudan Red 7B (IS)) and it helps to improve recoveries of the analytes for this complex matrix.

## Conclusion

Using the AutoMate-Q40 to automate the Sudan dye extraction method produced reliable results for the spiked samples. Automating this QuEChERS extraction shows the versatility of the AutoMate-Q40 and how it can be adapted to many different types of matrices. By automating the liquid handing, addition of salt/buffers, sample mixing, pipetting, and liquid level sensing using the patent pending VialVision™, the AutoMate-Q40 frees the scientist from a labor-intensive extraction method and exposure to unhealthy chemicals. The AutoMate-Q40 led to improved repeatability, a reduction in the likelihood of human error and the potential for significant labor savings.

Using the AutoMate-Q40, the automated QuEChERS extraction is a suitable extraction for illegal dyes from chili products. Precision and accuracy were assessed for the chili powder analyzed. Results for the automated procedure were well within the criteria set forth in this study: Average recoveries for the range of commodities were between 68% and 113% with good precision (ca. 5% RSD).

## References

1. 2004/92/EC: Commission Decision of 21 January 2004 on emergency measures regarding chili and chili products (notified under document number C[2004] 68) OJ L27/52 (30.1.2004)
2. Larsen, John Chr. "Legal and illegal colors" Trends in Food Science & Technology (2008), 19(Suppl. 1), S60-S65
3. www.sigmaaldrich.com for the structures of the compounds