A Rapid iMethod™ Test for the Quantification of Azo-Dyes in Food

iMethod™ Test

The Sudan dyes Sudan I, Sudan II, Sudan III and Sudan IV belong to the compound group of the Azo-dyes. Recent additions to this group of regulated dyes also include Para Red, Fast Garnet GBC, Orange II, Rhodamine B and Dimethyl Yellow. According to International Agency for Research on Cancer, Azo-dyes have been classified as potential carcinogenic substances. As such, Sudan dyes are banned as food additives in the European Union.

The following description outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethodTM Test for the Analysis of fifteen Azo-Dyes in Food, when using an AB SCIEX 3200 Series instrument. This method has also been developed and verified for use with 4000 Series instrumentation. More in depth sample preparation, and instrument parameter information is included as part of the standard operating procedure provided with the Azo-Dyes iMethod Test upon purchase.

General example sample preparation procedures are provided that are based upon a simple sample homogenization, centrifugation, extraction and dilution. Deuterated internal standards of Sudan I and Sudan IV at known concentrations are added during sample preparation to monitor sample recovery.

Results

An example chromatogram of the Azo Dyes is shown in Figure 1 with retention times and the MRM transitions used listed in Table 1. The superior sensitivity of the method is highlighted by the limits of detection shown. Signal-to-Noise varies depending on the ionization and fragmentation efficiency of each analyte. Thus Limits of Quantification (LOQ) are different for each.

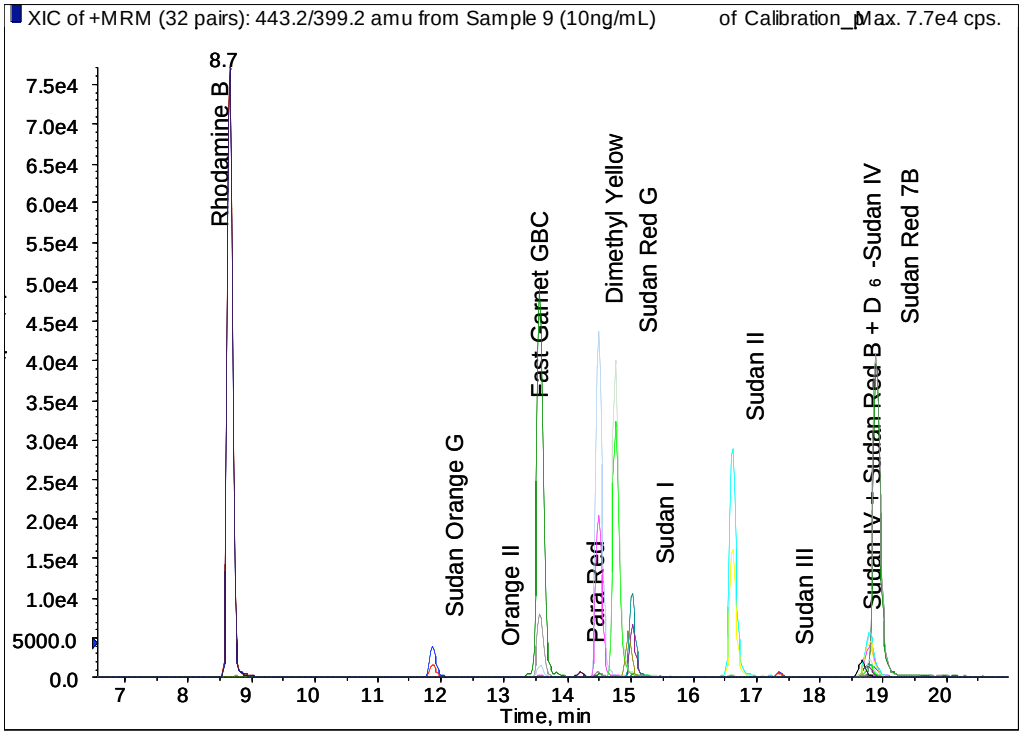

Figure 1: Chromatogram of Azo Dyes (10ng/mL) analyzed by LC/MS/MS in positive polarity

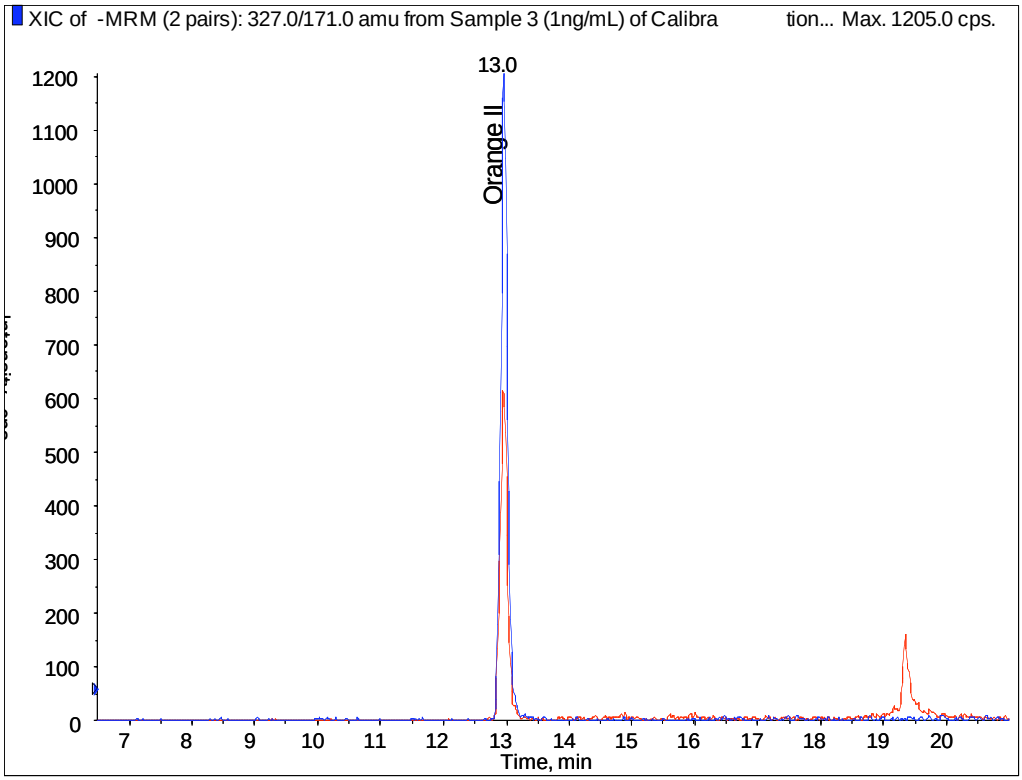

Figure 2: Chromatogram of Orange II (1ng/mL) analyzed by LC/MS/MS in negative polarity

Table 1. Multiple Reaction Monitoring (MRM) transitions, retention times, limit of detection (LOD) in solvent standards based on the qualifier MRM transition

Compound	MRM 1	MRM 2	RT (Mins)	LOD (ng/mL)
Dimethyl Yellow	226 / 120	226 / 105	14.5	<0.1
Fast Garnet GBC	226 / 91	226 / 107	13.5	0.1
Orange II (positive)	329 / 156	329 / 128	12.7	1
Orange II (negative)	327 / 171	327 / 80	12.7	0.2
Para red	294 / 156	294 / 128	14.2	0.1
Rhodamine B	443 / 399	443 / 355	8.6	<0.1
Sudan I	249 / 93	249 / 156	15.0	<0.1
Sudan II	277 / 121	277 / 106	16.6	<0.1
Sudan III	353 / 197	353 / 128	17.4	0.2
Sudan IV	381 / 224	381 / 225	18.8	0.5
Sudan Orange G	215 / 93	215 / 122	11.8	0.1
Sudan Red 7B	380 / 183	380 / 115	18.9	<0.1
Sudan Red G	279 / 123	279 / 108	14.7	0.5
Sudan Red B	381 / 224	381 / 225	18.8	0.2
D5-Sudan I	254 / 156		14.9	
D5-Sudan IV	387 / 106		18.7	

Calibration

The following example calibration curves using the calibrator, low and high level controls are provided as examples, showing the range and linearity expected for this assay.

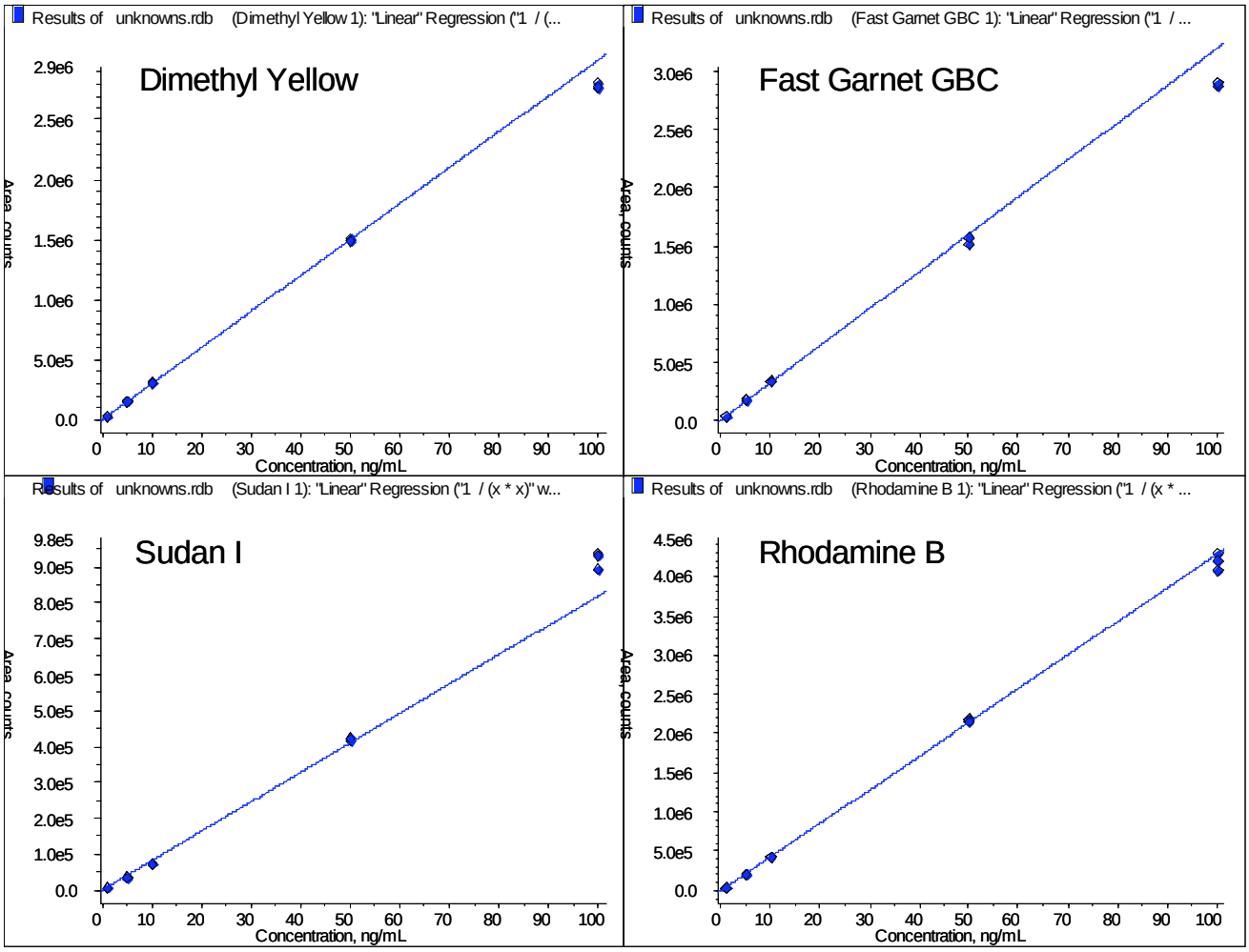

Figure 3: Representative calibration curves demonstrate linearity and range of assay

Please note that the results presented above were obtained using a single instrument and single set of standards and samples. Prior to production use, the method should be fully validated with real samples, and the results here may not be typical for all instruments. Variations in LC column properties, chemicals, environment, instrument performance and sample preparation procedures will impact performance, thus these results should be considered as informative rather than representative.

System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 3200 Series (3200 QTRAP® or API 3200™) or 4000 Series (4000 QTRAP® or API 4000™) LC/MS/MS System
- A Shimadzu Prominence 20A LC System with Reservoir tray and bottles, System controller CBM-20A, 100 µL mixer, 2 Isocratic pumps LC-20AD, 3 Channel degasser Autosampler SIL-20AC, Column oven CTO-20AC or Agilent 1100/1200 LC system with Binary pump G1312A (without static mixer), Well plate auto sampler, and Thermostated Column oven..
- Azo-Dye Standards (www.sigmaaldrich.com)
- Sudan I and Sudan IV Internal Standards (www.auftragssynthese.com)
- LC/MS Grade Water, Acetonitrile, Formic Acid and Ammonium formate
- A Phenomenex LUNA 5u C8, 150x2mm Column
- 1.5 mL Eppendorf Tubes
- A Centrifuge able to accommodate Eppendorf tubes and run at 14000 rpm
- Pipettes and standard laboratory glassware

Important Note

The purchase and use of certain of the chemicals listed above may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements. AB SCIEX is not responsible for user's compliance with any statute or regulation, or for any permit or approval required for user to implement any iMethod procedure.

Legal Acknowledgements/Disclaimers

AB SCIEX to provide the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. This method is provided for information purposes only. The performance of this method is not guaranteed due to many different potential variations, including instrument performance, tuning, and maintenance, chemical variability and procedures used, technical experience, sample matrices, and environmental conditions. It us up to the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

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