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Introduction

Azo compounds are formed from the diazotization of aromatic amines. These compounds have been used for decades in the textile, food, and paper industries to dye products a variety of colors. Some azo compounds have been shown to be mutagenic or carcinogenic, and may present a human health risk. As a result, these compounds are prohibited or regulated in food and other products. Therefore, it is important to develop a sensitive, accurate, and selective method to detect and quantify the levels of azo compounds in textile, food and paper products.



Figure 1. Molecular structure of Sudan I (left) and Sudan II (right) azo dyes.

Currently, the most common analytical methods used for azo compound detection include HPLC with UV or fluorimetric detection.² LC/MS/MS provides sensitive and selective detection, with the confidence of LC separation, as well as mass spectral precursor and product ions. In addition, many interferences are eliminated with the use of LC/MS/MS. The LC/MS/MS method outlined herein is sensitive, selective and quantitative, making it a useful tool for regulatory monitoring and quality assessment.

Instrumentation

- Varian 320-MS Triple Quadrupole LC/MS/MS with ESI source
- Varian 212-LC Binary Solvent Delivery Modules
- HTS PAL autosampler from CTC Analytics

Materials & Reagents

Standard azo dyes were purchased from Acros Organics, AppliChem (Darmstadt, Germany), Sigma-Aldrich (which included Sigma, Aldrich, Sigma-Aldrich and Fluka-branded standards) and Spectrum Chemical & Manufacturing Co. All solvents were Optima grade purchased from Fisher Scientific.

Sample Preparation

For each standard, 100 mg of sample was diluted into 1 L of HPLC grade methanol and then diluted to concentrations of 5, 10, 20, 50, 100, and 200 ppb. Orange (II) standard was prepared the same way in HPLC grade water.

Application Note 01506

Analysis of 15 Azo Dyes Using the Varian 320-MS Triple Quadrupole LC/MS/MS

HPLC Conditions

Column:	Pursuit [®] C18 (Varian Part	3 μm, No. A30	150 x 2.0 001150X0	mm ID 20)
Solvent A:	Water			
Solvent B:	Acetonitrile			
LC Program:	Time (min:sec)	% A	%B	Flow (µL/min)
	00:00	80	20	200
	01:00	80	20	200
	10:00	0	100	200
	25:00	0	100	200
	25:01	80	20	200
	32:00	80	20	200
ection Volume:	20 µL			

API Conditions

Ini

Ionization Mode:	ESI (positive/negative)
API Drying Gas:	35 psi at 400 °C
API Nebulizing Gas:	35 psi
Needle:	±4500 V
Shield:	±600 V

Table 1. MS/MS Transitions.

Compound	Q1	Q3 #1	Q3 #2	Capillary Voltage	CID #1	CID #2	Dwell Time(s)
p-Nitroaniline	139.1	92.9	75.9	52	13.0	27.0	0.05
Orange (II)	326.9	155.2	170.8	-68	28.5	22.5	0.05
Rhodamine B	443.2	355.3	399.2	120	45.0	38.0	0.05
Sudan Orange G	215.2	92.9	198.1	44	20.0	11.5	0.05
Fast Garnet GBC	226.1	90.9	121.0	52	14.5	17.5	0.05
Para Red	294.3	127.9	156.0	44	23.0	13.0	0.05
Dimethyl Yellow	226.3	76.9	119.9	52	14.5	27.0	0.05
Sudan Red G	279.1	123.0	248.1	40	13.5	10.0	0.05
Toluidine Red	308.3	127.9	156.0	40	26.5	13.5	0.05
Sudan I	249.0	128.0	156.0	52	22.5	12.5	0.05
Sudan II	277.0	156.0	120.0	48	12.5	10.5	0.05
Sudan III	353.0	156.0	128.0	76	15.5	29.5	0.05
Sudan IV	381.0	224.0	106.0	84	15.5	27.0	0.05
Sudan Red 7B	380.1	169.1	183.1	44	16.5	11.5	0.05
Sudan Black B	457.1	142.0	246.1	88	37.5	20.0	0.05

1 1 2		CECT - CENSION AND CONTRACT OF A
,	4-Nitroani	line #2
2 82 84 14	4-Nitroanil	ine #1
1111	Orange (II)	#1 (-ESI)
2002020	Orange (II)	#2 (-ESI)
1 1 1 1 1 1	Rhodam	ine B #1
	Rhodam	ine B #2
	Sudan O	Prange G #1
2 2 2 2	Sudan (Drange G #2
	Fa	st Garnet GBC #1
2 2 2 2 2 2 2	Fa	ast Garnet GBC #2
	Pa	ara Red #1
	Pa	ara Red #2
	Di	imethyl Yellow #1
5	Di	imethyl Yellow #2
	S	udan Red G #1
		THE REPORT OF THE PARTY AND ADDRESS OF THE PARTY AND ADDRESS OF THE PARTY ADDRESS OF
	S	udan Red G #2
	Sudan I #1	udan Red G #2
	Sudan I #1	udan Red G #2
	Sudan I #1 Sudan I #2 Sudan II #2	udan Red G #2
	Sudan I #1 Sudan I #2 Sudan II #2 Sudan II #1	
<u> </u>	Sudan I #1 Sudan I #2 Sudan II #2 Sudan II #1 Sudan III #2	udan Red G #2
	Sudan I #1 Sudan I #2 Sudan II #2 Sudan II #1 Sudan III #2 Sudan III #2 Sudan III #1	udan Red G #2
	Sudan I #1 Sudan I #2 Sudan II #2 Sudan II #2 Sudan III #1 Sudan III #1 Sudan IV #2	udan Red G #2
<u></u>	Sudan I #1 Sudan I #2 Sudan II #2 Sudan II #2 Sudan III #2 Sudan III #2 Sudan III #1 Sudan IV #2 Sudan IV #1	
<u> </u>	Sudan I #1 Sudan I #2 Sudan II #2 Sudan II #2 Sudan III #1 Sudan III #1 Sudan IIV #2 Sudan IV #1 Toluidine Red #1	udan Red G #2
<u> </u>	Sudan I #1 Sudan I #2 Sudan II #2 Sudan II #2 Sudan III #2 Sudan III #1 Sudan III #1 Sudan IV #2 Sudan IV #1 Toluidine Red #1 Toluidine Red #2	
<u></u>	Sudan I #1 Sudan I #2 Sudan II #2 Sudan II #2 Sudan III #2 Sudan III #1 Sudan IV #2 Sudan IV #2 Sudan IV #1 Toluidine Red #1 Toluidine Red #2 Sudan Red 7B #1	
<u>22222</u>	Sudan I #1 Sudan I #2 Sudan II #2 Sudan II #2 Sudan III #2 Sudan III #1 Sudan IV #2 Sudan IV #2 Sudan IV #1 Toluidine Red #1 Toluidine Red #1 Sudan Red 7B #1 Sudan Red 7B #2	
22222 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Sudan I #1 Sudan I #2 Sudan II #2 Sudan II #2 Sudan III #2 Sudan III #1 Sudan III #1 Sudan IV #2 Sudan IV #1 Toluidine Red #1 Toluidine Red #1 Sudan Red 7B #1 Sudan Red 7B #2 Sudan Black #1	

Figure 2. Mass chromatogram for a 50 ppb injection of the azo dyes. The #1 and #2 labels refer to the transitions in Table 1.

Results & Discussion

The MS parameters were optimized for each of the dyes by infusing a 1 ppm standard solution at 20 μ L/min while supplying a flow of 50:50 mix of mobile phases A and B at 180 μ L/min. This gave a total flow of 200 μ L/min of approximately 100 ppb standard mix. Once the MS parameters were optimized for each of the dyes, 20 μ L injections were made from 5 ppb to 200 ppb. Figure 2 shows the stacked chromatogram for all 32 transitions of the 15 azo dyes.

Using a single injection for each calibration point, most calibration curves were found to be linear from 5 ppb to 200 ppb. Figure 3 shows the calibration curve for Sudan Black B with %RSD at 8.55% and r² value of 0.9999.

Calibration Curve Report File: ... + 'blue' sudans msms (32 ions) fast 80-20 start 50ms - quant.mth Detector: Quad Mass Spec, Address: 42



Figure 3. Calibration curve for Sudan Black B from 5 ppb to 200 ppb. RSD is 8.55%, r^2 value is 0.9999.

Conclusion

The Varian 320-MS Triple Quadrupole Mass Spectrometer is able to detect and quantify 15 azo dyes over a wide calibration range. This method is rapid, sensitive and quantitatively accurate, and is performed in a single analytical run with a $20-\mu$ L injection.

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