

# Characterization of Di- and Tri-substituted Sulfonated Azo and Acid Dyes by LC/ESI/MS/MS, LC/EI/MS and LC/CI/MS

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## INTRODUCTION

Azo dyes have been used for a variety of purposes in the textile, colorant, and food industries. It is estimated that about 65 million pounds of sulfonated azo dyes are used annually by the textile industry. Azo dyes are also found as colorants in water-soluble markers and in certain types of processed foods. The general structure of an azo dye is Ar-N=N-Ar'. The addition of sulfonic acid groups to the aromatic rings enhances the solubility, but imparts a low volatility to the molecule. This low volatility significantly reduces, if not eliminates, the information that can be obtained from the standard electron-impact mass spectrometry techniques.

In order to gain a better understanding of the sulfonated dyes with regards to their mass spectral detection and characteristic fragmentation patterns, this study analyzed seven disulfonated and trisulfonated azo dyes using liquid chromatography electrospray ionization (ESI), liquid chromatography electron impact (EI), and liquid chromatography chemical ionization (CI) mass spectrometry. LC/ESI/MS/MS was conducted and recorded in alternating positive and negative modes on the commercially obtained dyes after chromatography with isocratic 35% acetonitrile: water with 0.1% formic acid on a C-18 column. LC/EI/MS or LC/CI/MS data was obtained under the same chromatographic conditions by utilizing a particle beam interface attached to an Agilent 5973 MSD.

## SAMPLE PREPARATION FOR MASS SPECTROSCOPY

The dyes were dissolved in deionized water for both the CI and EI experiments to concentrations of 10, 100, 1000, 6000 ppm. For ESI the dyes were dissolved in deionized water with 0.1% formic acid present to the final concentrations of 10 and 100 ppm. The dyes were extracted from the water-soluble markers by immersing the tip in 1 mL of water for 30 seconds.

## INSTRUMENTATION – PARTICLE BEAM LC/EI/MS AND LC/CI/MS INTERFACE

For particle beam LCMS, the system included the following components. The liquid chromatograph used was an Agilent Model 1100 modular system with quaternary pump, vacuum degasser, 100 vial autosampler and variable wavelength detector. The samples were analyzed by direct injection without a HPLC column. The Genesis II particle beam interface (CSS Analytical Co Inc.) was attached to an Agilent 5973 MSD so that samples can be analyzed by LC/MS with electron impact and chemical ionization. The Genesis II is an improved particle beam interface, which delivers a higher amount of analyte to the ion source, when compared to previous commercial interfaces. The mass spectrometer used was an unmodified Agilent 5973 Mass Selective Detector (Agilent Technologies, Inc., Palo Alto California) with turbo molecular pump. The Agilent 5973 is a bench top quadrupole mass spectrometer with mass range of 1.6 to 800 mass units, 10,000 volt HED, and is available with EI or EI/CI capabilities. The CI experiments were conducted in the positive CI mode utilizing methane as the ionization gas.

## INSTRUMENTATION – HPLC/DAD/ESI-MS/MS AND MRM ANALYSES

LC/ESI-MS/MS experiments were performed on an Agilent MSD XCT ion trap mass spectrometer (Palo Alto, CA) equipped with an electrospray ionization (ESI) interface, 1100 HPLC, a DAD detector, and Chemstation software. The column used was a 150 x 0.5 mm i.d., Zorbax SB-C18 5  $\mu$ m (Agilent, Palo Alto, CA). Solvents were (A) 0.1% formic acid/ 99.9% water (v/v) and (B) 0.1% formic acid/ 99.9% acetonitrile (v/v). Isocratic elution was preformed with 35% B. Flow rate was 5.000  $\mu$ L/min, injection volume was 0.5 $\mu$ L, and column temperature was 25  $^{\circ}$ C. The ESI parameters were as follows: nebulizer, 15 psi; dry gas (N<sub>2</sub>), 5.00 L/min; dry temperature, 325  $^{\circ}$ C; trap drive, 66.3; skim 1, 40 V; lens 1, -5.00 V; octopole RF amplitude, 200 Vpp; capillary exit, 136.0 V. The ion trap mass spectrometer was operated in alternating ion mode scanning from m/z 100 to m/z 2200 at a scan resolution of 13000 amu/s. Trap ICC was 30000 units and maximal accumulation time was 200000  $\mu$ s. MS-MS was operated at a fragmentation amplitude of 1.2 V, and threshold ABS was 3,000,000 units.

ESI-MRM experiments were conducted on the Agilent MSD XCT ion trap by direct infusion utilizing a syringe pump set at 300  $\mu$ L/hour. The ESI parameters were as follows: nebulizer, 15 psi; dry gas (N<sub>2</sub>), 5.00 L/min; dry temperature, 325  $^{\circ}$ C; trap drive, 66.3; skim 1, 40 V; lens 1, -5.00 V; octopole RF amplitude, 200 Vpp; capillary exit, 136.0 V. The ion trap mass spectrometer was operated in positive ion mode scanning from m/z 100 to m/z 900 at a scan resolution of 13000 amu/s. Trap ICC was 30000 units and maximal accumulation time was 300000  $\mu$ s. MS-MS was operated at a fragmentation amplitude of 0.8 V, and threshold ABS was 3,000,000 units.

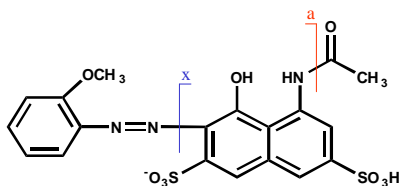
The MRM was configured to monitor for molecular weights 407, 466, 494, 507, 558, 668, and 747, respectively corresponding to FD&C Yellow #6, acid yellow 23, acid violet 12, acid yellow 17, acid red 52, acid green 3, and acid blue 9. The width was set to a value of 4 and the fragmentation was off. Initially each individual dye was infused and the utility of the MRM in detecting the dyes was assessed. Subsequently an orange marker was selected for analysis since it was likely a mixture of a red and yellow dye.

## MATERIALS

Acid Violet 12 (6625-46-3) was obtained from TCI America (Portland, OR). The remaining dyes F.D. & C. Yellow #6 (2783-94-0), Acid Yellow 17 (6359-98-4), Acid Yellow 23 (1934-21-0), Acid Blue 9 (3844-45-9), Acid Green 3 (4680-78-8), and Acid Red 52 (3520-42-1) were obtained from Sigma Aldrich (St. Louis, MO). Burdick and Jackson HPLC grade water and acetonitrile were purchased from VWR Scientific (West Chester, PA).

FIGURE 1. PROPOSED FRAGMENTATION ROUTES BY ESI

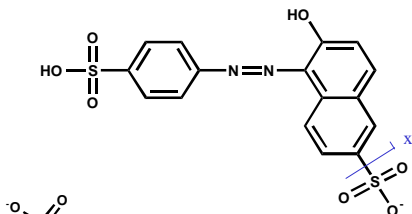
Acid Violet 12  
(Pontacyl Carmine 2B)  
MW 495.48



Positive mode  
Negative mode

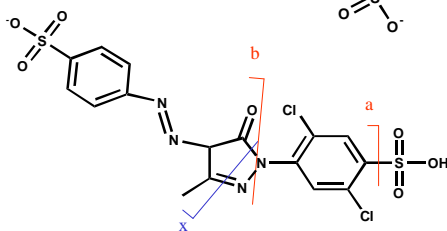
496.5  $\xrightarrow{a}$  454.0  
494.3  $\xrightarrow{x}$  358.9

FD&C Yellow #6  
(Sunset Yellow FCF)  
MW 408.41



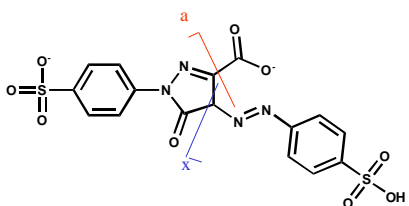
407.1  $\xrightarrow{x}$  327.1

Acid Yellow 17  
MW 507.32



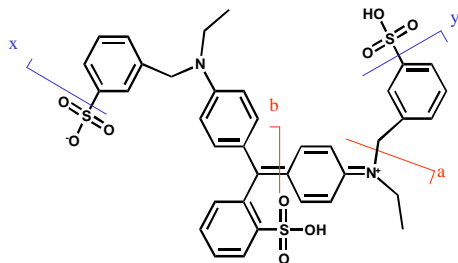
507.0  $\xrightarrow{a}$  425.1  $\xrightarrow{b}$  266.0  
505.0  $\xrightarrow{x}$  252.0

Acid Yellow 23  
(Tartrazine)  
MW 468.42



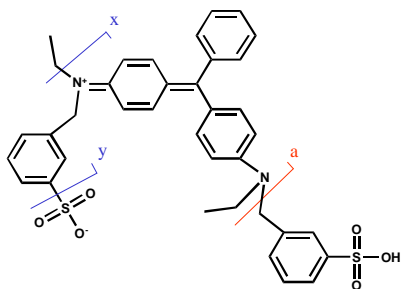
469.1  $\xrightarrow{a}$  425.1  
467.0  $\xrightarrow{x}$  197.9

Acid Blue 9  
(Erioglaucine)  
MW 748.88



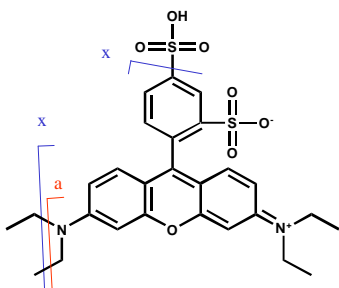
749.2  $\xrightarrow{a}$  579.2  $\xrightarrow{b}$  458.1  
747.2  $\xrightarrow{x}$  667.3  $\xrightarrow{y}$  587.3

Acid Green 3  
(Guinea Green)  
MW 669.83



669.3  $\xrightarrow{a}$  499.3  
667.1  $\xrightarrow{x}$  639.2  
667.1  $\xrightarrow{y}$  587.3

Acid Red 52  
(Sulforhodamine B)  
MW 558.67



559.2  $\xrightarrow{a}$  [C3H8] 515.1  
557.3  $\xrightarrow{a}$  [C3H8] 513.2  
557.3  $\xrightarrow{y}$  477.3

## RESULTS

The dyes selected for the study included Acid Violet 12, F.D. & C. Yellow #6, Acid Yellow 17, Acid Yellow 23, Acid Blue 9, Acid Green 3, and Acid Red 52. These dyes are commonly found in water-soluble markers manufactured by Crayola™ and in one marker collection are mixed to yield 50 different colors.<sup>2</sup> Our preliminary work has allowed us to develop predictive mechanisms of azo dye fragmentation that could prove useful in identifying the azo and acid dyes under similar analytical conditions in clothing, colorants, foodstuffs, or as environmental bioaccumulants.

Our initial experiments on analyzing the dyes by LC/EI and LC/ESI utilized the markers from a Crayola™ 50 marker pack. The dye or combination of dyes in each marker comprises a minor percentage of the marker composition. The majority of the components are stabilizers, water, alcohol, and an anionic dye blocker. The dye blocker is an aromatic organic sulfonate which is based on a sulfonated phenol and/or a sulfonated naphthalene condensation product with formaldehyde. This dye blocker existed in numerous polymeric forms and could be observed at seemingly all points in the chromatographic separation. The prevalence of the polymeric dye blocker and its sodium adducts in additions from +1 to +10 sodium ions made the discernment of the azo or acid dyes which are sulfonated benzene or naphthalene ring systems impractical.

Dye standards were obtained from Sigma Aldrich and TCI America to acquire mass spectral data on the pure compounds. The standards had values from 40 to 90% dye content and presented similar issues to the markers. The dyes were analyzed by LC/ESI and MS/MS data was obtained in alternating positive and negative modes. Several common fragments were observed and are shown in FIGURE 1.

**ESI mode with LC.** The parent mass ions generated under ESI conditions for the dyes were consistently in higher abundance in the positive mode over the negative mode for all but one of the dyes (F.D. & C. Yellow #6). This was presumably due to the lack of a sufficiently basic nitrogen to accept the H<sup>+</sup>, since the F.D. & C. Yellow #6 possesses nitrogen only in the azo functionality. Sodium adducts (Na<sup>+</sup>) were observed for the dyes corresponding maximally to the number of sulfite groups present, but more frequently to M+Na.

**EI mode.** The analysis of the dyes under the EI conditions yielded very limited useful data for identification. We were unable to find any evidence of parent ion formation and the low molecular weight fragments that did form were of little use in distinguishing the dyes from one another. Previous work done by Straub (1992) utilizing a particle beam interface for LC/EI was conducted on 14 azo dyes (none of which were examined in our work). Only four of the dyes examined had MW in excess of 400 and only one of these was able to yield parent ion data. The remaining 10 dyes did give parent ions, but the base peaks in each of the cases were frequently low molecular weight fragments. Of the seven dyes we examined all had MWs in excess of 400 and the additional complexity of the structures and resultant instability in LC/EI/MS seemingly prevented useful analysis.

**CI mode.** The chemical ionization experiments in the positive mode with methane failed to generate significant analyzable data for any of the dyes examined.

**ESI with MRM.** The experiments in which the dyes were analyzed by direct infusion into the Agilent ion trap using ESI with MRM were successful in obtaining useful data for the seven dyes tested. The MRM allows for the simultaneous monitoring of 10 different ions. Using the multiple reaction monitoring/selective ion monitoring feature of the ion trap MS we were able to easily locate the parent ion for each infused dye and subject them to fragmentation. A single-blind experiment was conducted to determine if the instrument operator could ascertain the identity of a dye while using the MRM to monitor for all seven dyes simultaneously. The dyes were successfully analyzed singly and in combination under these conditions. In one experiment an orange marker was extracted into water and the components Acid Red 52 and Acid Yellow 17 were easily detected despite the presence of the numerous stabilizers and anionic dye blockers which had presented such difficulty when examining the dyes using LC separation.

## CONCLUSIONS

The detection of the dyes through LC/ESI/MS/MS and MRM was successful in yielding parent ion information on each of the azo and acid dyes. Several structurally important fragment ions were obtained in positive and negative mode. The loss of SO<sub>3</sub> was observed in the negative mode for the dyes whereas some fragments such as CH<sub>2</sub>CH<sub>3</sub>, and SO<sub>3</sub>C<sub>6</sub>H<sub>4</sub>CH<sub>2</sub> have been observed to form in either mode. These fragments and others that were observed will help to characterize and identify sulfonated azo dyes found in clothes, markers, and foodstuffs. Our experimentation with the dyes by LC/EI/MS utilizing a particle beam interface yielded principally low molecular weight structural fragments with no parent mass ions detected even in minor abundance. While volatilization of the dyes was obtained under the experiment conditions, the absence of parent mass ions seems to indicate that the energy in the EI proved to be too high for the analysis despite attempts with 70 and 35 keV. While we were unable to generate any parent ions in the positive CI mode with methane, additional work with the LC/CI/MS and particle beam interface will be carried out to investigate the utility of this softer ionization technique in the negative mode to generate parent mass ions.

In summary, an evaluation of our experiments showed the following:

1. The LC/ESI/MS and MRM works well for the detection of the dyes in complex mixtures.
2. The LC/EI/MS while it was able to yield some fragmentation data with the pure compounds did not provide useful identification data for the intact molecules due to the absence of parent ions.
3. LC/EI/MS with Particle Beam sample introduction is capable of providing fragmentation information on molecules inaccessible to other techniques.

## FUTURE WORK

Our intentions are to utilize the LC/ESI method with MRM to generate complete fragmentation data to permit the detection and identification of the intact dyes and their degradation products.

## REFERENCES

1. Thermospray, particle beam and electrospray liquid chromatography-mass spectrometry of azo dyes. Rolf Straub, et. al., Journal of Chromatography, 1992, 627, 173-186.
2. United States Patent 5,968,241. Andree F. Santini and Richard E. Miller. Washable coloring composition. 1999.