Acid Blue 9

Method no.:	PV2129
Matrix:	Air
Target Concentration:	0.2 mg/m^3. There is no OSHA PEL for acid Blue 9. Neither NIOSH nor ACGIH has a recommended standard for Acid Blue 9. For the purpose of this study, the target concentration has been arbitrarily set at 0.2 mg/m3. It represents 100 x the detection limit for the proposed method.
Procedure:	Collection on a glass fiber filter, extraction with methanol/water (1:1), and analysis by high performance liquid chromatography (HPLC) with variable wavelength detector at 650 nm.
Recommended Air Volume and Sampling Rate:	100 L at 1 Lpm
Detection Limit of the Overall Procedure Based on Recommended Air Volume:	0.002 mg/m^3
Status of Method:	This method has been only partially validated and is presented for information and trial use.

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1. General Discussion

1.1. Background

1.1.1. History of Procedure.

Recently, the OSHA Analytical Laboratory received a set of field samples requesting analysis for Acid Blue 9. The air samples had been collected on glass fiber filters, at 1 Lpm for a total of about 90 liters air. This report describes the analytical procedure developed and the preliminary validations of the sampling method.

Acid Blue 9 is a widely used food dye. There has been many schemes proposed for the qualitative analysis of food dyes, most of which depended on paper and thin-layer chromatography. Less attention has been given to the quantitative analysis of dyes. Some of the methods attempted were: (a) comparison of spot intensities on TLC plates with those of a range of standards, (b) spectrophotometric quantitation, (c) titration with titanous chloride solution, and (d) electrophoresis on polyacrylamide gel. More recently, HPLC has been applied for dye analysis, using anion-exchange columns or, more satisfactorily, by ion-pairing. Paired-ion HPLC affords a means of separating a mixture of food dyes in a single run (Ref. 5.2.). Preliminary search did not reveal an air sampling method for Acid Blue 9. Judging from its physical properties, glass fiber filters may be a suitable collection medium.

1.1.2. Toxic Effects.

(This section is for information only and should not be taken as the basis of OSHA policy.)

Acid Blue 9 is carcinogenic in rats after its subcutaneous injection: it produced fibro sarcomas following repeated injections. It also produced an increased incidence of kidney tumors in mice after its oral administration (Ref. 5.1.).

1.1.3. Potential Workplace Exposure.

Acid Blue 9 is an FDA certified food dye and is used in such products as gelatin desserts, ice cream and sherbets, carbonated beverages, dry drink powders, candy and confectionary products when they do not contain oils and fats, bakery products and cereal, puddings, aqueous drug solutions, tablets, capsules, bath salts, and hair rinses (Ref. 5.3.). Acid Blue 9 has been produced in the U.S. for over sixty years. In 1975, three U.S. companies produced 622,000 Kg of the general dye grade, and another four companies produced 56,000 Kg of he food, drug, and cosmetic grade (Ref. 5.1.). Preliminary literature searches did not reveal any estimate on the extent of worker exposure.

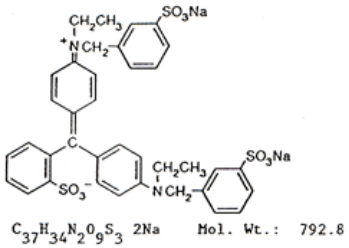
1.1.4. Physical Properties

Color Index Names: Acid Blue 9, Food Blue 2
Color Index Number: 42090
CAS Reg. Number:2650-18-2 (3844-45-9)
Chem. Abstr. Names:
N-Ethyl-N-(4[(4-(ethyl[(3-sulfophenyl)methyl]amino) phenyl)-(2-sulfophenyl)methylene]-2, 5-cyclohexadien-1-ylidene)3-sulfobenzenemethanaminium hydroxide inner salt, disodium salt; C.I. Acid Blue 9, disodium salt; D and C Blue No.1; D and C Blue No.4; ethyl(4-(p[ethyl (*m*-sulphobenzyl)amino]-α -(o-sulphophenyl)benzylidene)-2,5-cyclohexadiene-l-ylidene)- (*m*-sulphobenzyl) ammonium hydroxide inner salt, disodium salt; FD and C Blue 1; FD and C Blue. No.1; FDC Blue No.1; Acid Sky Blue A; Acilan Turquoise Blue AE; A. F. Blue No.1; Aizen Brilliant Blue FCF; Aizen Food Blue No.1; Alphazurine; Alphazurine FG; Alphazurine FGND; Amacid Blue FG; Amacid Blue FG Conc; 1206 Blue, 11388 Blue; Blue Dye Number 1 food additive; Brilliant Blue; Brilliant Blue FCF; Brilliant Blue Lake; Bucacid Azure Blue; Calcocid

Blue EG; Calcocid Blue 2G; Canacert Billiant Blue FCF, Cogilor Blue 512.12; Cosmetic Blue Lake; Dispersed Blue 12195; Disulphine Lake Blue EG; Dolkwal Brilliant Blue; Edicol Blue CI 2; Edicol Supra Blue E6; Erioglaucine ; Erioglaucine A; Erioglaucine E; Erioglaucine G; Eriosky Blue; Fenazo Blue XI; Fenazo Blue XR; Food Blue 1; Hexacol Brilliant Blue A; Hidacid Azure Blue; Intracid Pure Blue L; Kjtoc Blue AR; Kiton Pure Blue L; Maple Brilliant Blue FCF; Merantine Blue EG; Neptune Blue BRA; Concentration; Patent Blue AE; Patent Blue 2Y; Peacock Blue X-1756; Usacert Blue No.1; Xylene Blue VSG.

Appearance: Reddish-violet powder or granules with a metallic luster.
Spectroscopy Data: λ max 630 nm.

Chemical Formula and Molecular Weight:



Solubility: Soluble in water and ethanol; insoluble in vegetable oils.

1.2. Limit Defining Parameters

1.2.1. Detection Limit of the Analytical Procedure

The detection limit of the analytical procedure is 0.83 ng Acid Blue 9 per injection. This is the amount of analyte which will give a peak whose height is approximately five times the amplitude of the baseline noise. See Figure 1.

1.2.2. Detection Limit of the Overall Procedure

The detection limit of the overall procedure is estimated to be 0.2 µg per sample or 0.002 mg/cu m based on the recommended air volume, assuming 100% recovery from the sampling device. The recovery test at this level has not been performed.

1.2.3. Sensitivity

The sensitivity of the analytical procedure over a concentration range of 0.395 to 11.9 µg/mL is 19,280 area units per µg/mL of Acid Blue 9. The sensitivity is determined by the slope of the calibration curve. See Figure 2.

1.3. Advantages

The analytical procedure is rapid, sensitive, and reproducible.

1.4. Disadvantages

The method has not been fully validated.

2. Sampling Procedure

2.1. Apparatus

2.1.1. An air sampling pump with a flow rate which can be calibrated to within ±5% of the recommended 1 Lpm flow rate while the sampler is in line.

2.1.2. Glass fiber filter, 37-mm diameter, Gelman Type A, or equivalent.

2.1.3. Filter holder for 37-mm filters, Millipore M000037AO, or equivalent.

2.2. Sampling Technique

2.2.1. Assemble the filter in the two-piece cassette holder and close firmly. The filter is supported by a backup pad. Secure the cassette holder together with tape.

2.2.2. Attach the outlet of the filter cassette to the personal sampling pump inlet with flexible tubing.

2.2.3. Air being sampled should not pass through any hose or tubing before entering the filter cassette.

2.2.4. A sample size of 100 liters is recommended. Sample at a flow rate of 1.0 liter/minute. The flow rate should be known with an accuracy of ±5%.

2.2.5. With each batch of samples, submit a blank filter from the same lot of filters used for sample collection. This filter must be subjected to exactly the same handling as the samples except that no air is drawn through it. Label this filter as the blank.

2.2.6. The cassette should be shipped in a suitable container designed to prevent damage in transit. The samples should be shipped to the laboratory as soon as possible.

2.2.7. A sample of the bulk material should be submitted to the laboratory in a glass container with a Polyseal cap. Never transport, mail, or ship the balk sample in the same container as the sample or blank filter.

2.3. Retention Efficiency

Two glass fiber filters were spiked with 1.1.5 µg of Acid Blue 9. Humid air (87% relative humidity) 140 liters was drawn through the filters at 1 Lpm. The average recovery of the two filters was 101%.

Sample	Spiked Amount	Treatment	Peak Height	Recovery
YC5	41.5 µg on GFF	140 L humid air	138.0 mm	103.4%
YC6	41.5 µg on GFF	140 L humid air	131.5 mm	98.5%
YC7	41.5 µg; control	none	131.0 mm	----
YC8	41.5 µg; control	none	136.0 mm	----

Average recovery 100.9%

2.4. Extraction Efficiency

The average extraction efficiency from the glass fiber filters spiked with 41.5 µg of Acid Blue 9 was 95.9%.

Sample	Spiked Amount	Peak Height	Recovery
YC3	41.5 µg on GFF	126.5 mm	94.8%
YC4	41.5 µg on GFF	129.5 mm	97.0%
YC7	41.5 µg; control	131.0 mm	----
YC8	41.5 µg; control	136.0 mm	----

Average recovery 95.9%

2.5. Storage

Two glass fiber filters were spiked with 41.5 µg of Acid Blue 9 and stored at room temperature in the dark for two days. The average recovery was 100.2%.

Sample	Spiked Amount	Storage Days	Peak Height	Recovery
YC3	41.5 µg	0	126.5 mm	94.8%
YC4	41.5 µg	0	129.5 mm	97.0%
YC7	41.5 µg	control	131.0 mm	----
YC8	41.5 µg	control	136.0 mm	----
YC1	41.5 µg	2	168.0 mm	105.0%
YC2	41.5 µg	2	157.0 mm	98.1%
YC7	41.5 µg	control	162.0 mm	----
YC8	41.5 µg	control	158.0 mm	----

2.6. Recommended Air Volume and Sampling Rate

2.6.1. The recommended air volume is 100 liters.

2.6.2. The recommended sampling rate is 1 Lpm.

2.7. Interferences

There are no known interferences associated with the sampling procedure.

2.8. Safety Precautions

2.8.1. Attach the sampling equipment to the worker in such a manner that it will not interfere with work performance or safety.

2.8.2. Follow all safety practices that apply to the work area being sampled

3. Analytical Method

3.1. Apparatus

3.1.1. High performance liquid chromatograph equipped with pump, sample injector, variable wavelength detector, chart recorder, and other necessary hardware.

3.1.2. HPLC reverse phase C18 analytical column. Dupont Zorbax ODS column was used for this study.

3.1.3. An electronic integrator or other suitable method to measure detector response.

3.1.4. Microliter syringe or automatic sampling device for making sample injections.

3.1.5. Volumetric flasks of convenient sizes for preparing standards.

3.1.6. Shaking device for extraction of samples.

3.2. Reagents

3.2.1. Acid Blue 9 (Erioglaucine)

3.2.2. Tetrabutylammonium phosphate, reagent grade

3.2.3. Methanol, HPLC grade

3.2.4. Water, HPLC grade

3.2.5. Phosphoric Acid

3.3. Sample Preparation

3.3.1. Remove the filter form the cassette clean tweezers and place it in a 20-mL scintillation vial.

3.3.2. Add 5 mL of methanol/water (1:1) to the vial and cap it.

3.3.3. Shake the vials vigorously on a shaker for 30 minutes.

3.4. Standard Preparation

3.4.1. Standard of Acid Blue 9 is prepared by dissolving 8 to 12 mg (accurately weighed) of Acid Blue 9 in water in a 10-mL volumetric flask and making it to volume.

3.4.2. Dilute to the working range of 0.1 to 12 µg/mL with water.

3.4.3. Store standards in dark bottles under refrigeration.

3.5. Analysis

3.5.1. HPLC Conditions

Column:	Zorbax ODS (25 cm x 4.6 mm)
Mobile phase:	55% methanol, 45% water, 0.005 *M* tetrabutylammonium phosphate
Flow Rate:	1.0 mL/minute
Variable Wavelength Detector:	650 nm
Injection Volume:	20 µL
Retention Time:	7.8 minutes

3.5.2. Chromatogram

See Figure 1.

3.5.3. Peak magnitude is measured by electronic integrator or other means.

3.5.4. An external standard procedure is used to prepare a calibration curve from the analysis of at least three different concentrations from two separate weighings.

3.5.5. Bracket the sample with analytical standards.

3.6. Interferences (Analytical)

3.6.1. Any collected compound that has the same LC retention time as analyte and absorbs at 650 nm is an interference.

3.6.2. HPLC parameters may be varied to circumvent most interferences.

3.6.3. Retention time alone is not proof of a chemical identity. Confirmation by other means should be sought when possible.

3.7. Calculations

3.7.1. The integrator value in area units for each standard is plotted against its concentration in µg/mL and a calibration curve using the best fit straight line through the points is obtained.

3.7.2. Sample concentration is calculated from the calibration curve.

3.7.3. The air concentration of Acid Blue 9 for a sample is calculated by the following equation:

$$mg/m^3 = \frac{(\mu g/mL \text{ in sample})(\text{extraction volume, mL})}{(\text{Air volume, L})}$$

3.8. Safety Precautions

3.8.1. Confine the use of solvents to a fume hood.

3.8.2. Wear safety glasses in all laboratory areas.

4. Recommendations for Further Study

4.1. Preparation of pure standard

The commercially available Acid Blue 9 is not pure. The U.S. specification for the food grade is 85% minimum. Purification of the standard should be attempted either by preparative TLC or preparative HPLC.

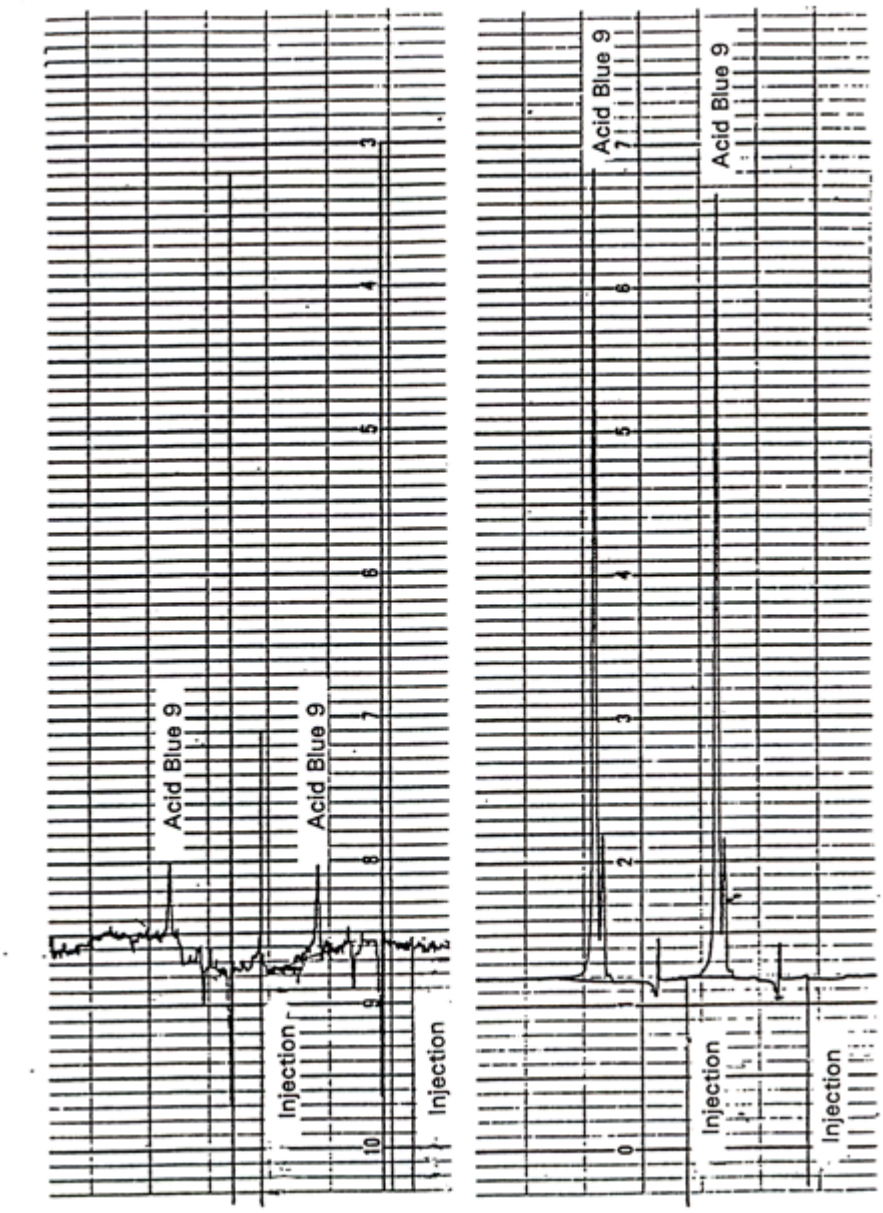


Figure 1. Chromatogram of Acid Blue 9 at Target Concentration and at Detection Limit.

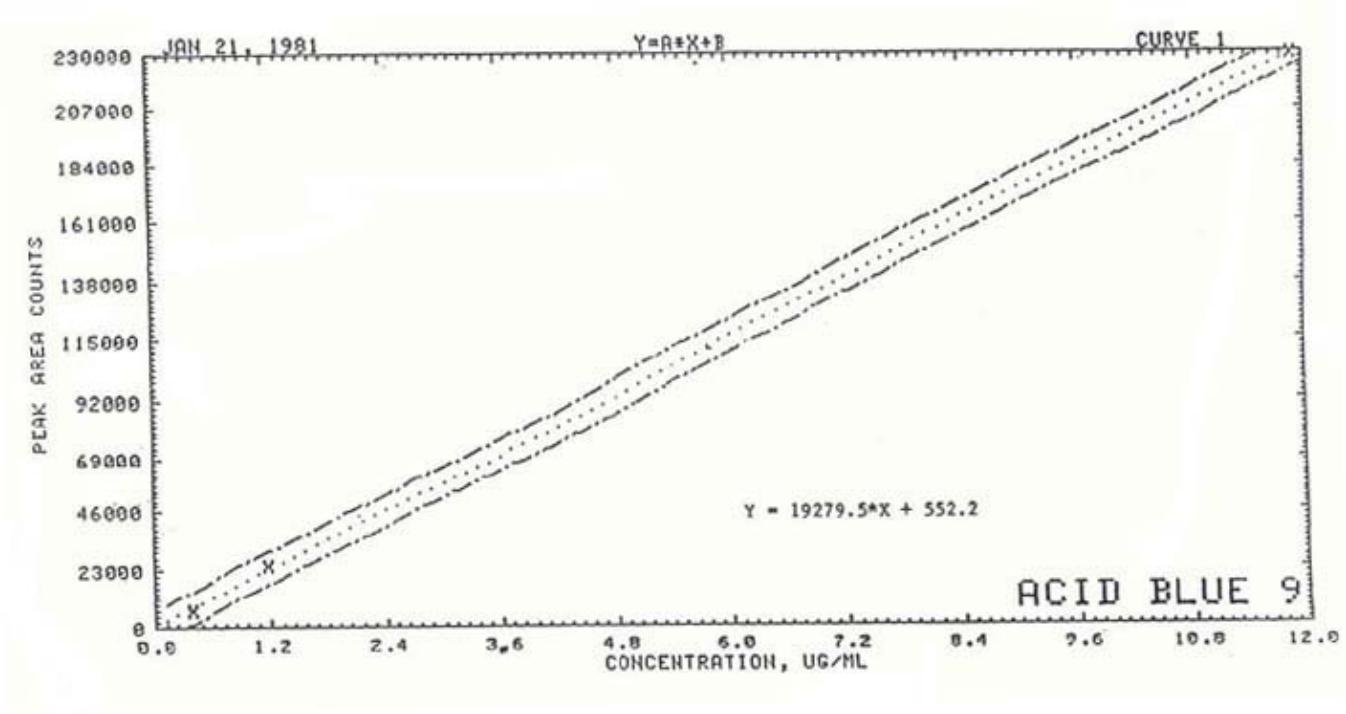


Figure 2. Calibration of Acid Blue 9.

5. References

5.1. WHO, International Agency for Research on Cancer, *IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Some Aromatic Amines and Related Nitro Compounds -- Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals.* Vol. 16, pp. 171-86.

5.2. J. Chudy, N.T. Crosby, and I. Patel, J. Chromatogr., 154, (1978), p 306-312.

5.3. A Standen, ed., *Kirk-Othmer Encyclopedia of Chemical Technology*, Second Ed., Vol. 5, pp. 865-66. Interscience Publishers, New York, N.Y., 1963.