RHODAMINE B

Method no.:	PV2072
Matrix:	Air
Target Concentration:	0.05 mg/m³ (arbitrary) There is no OSHA permissible exposure level (PEL) or ACGIH threshold limit value (TLV) for rhodamine B.
Procedure:	Samples are collected by drawing known volumes of air through glass fiber filters. Samples are extracted with methanol and analyzed by high performance liquid chromatography (HPLC) using ultraviolet (UV) or fluorescence detectors.
Recommended air volume and sampling rate:	240 L at 1.0 L/min
Detection limit of the overall procedure (based on the recommended air volume and the analytical detection limit):	0.28 µg/m³
Status of method:	Stopgap method. This method has been partially evaluated and is presented for information and trial use only.

Date: February 1989 Chemist: Duane Lee

Carcinogen And Pesticide Branch
OSHA Analytical Laboratory
Salt Lake City, Utah

1. General Discussion

1.1 Background

1.1.1 History of procedure

The OSHA Analytical Laboratory received a set of air samples requesting the analysis of rhodamine B. The samples had been collected on polyvinyl chloride filters (FWS-B) with air volumes around 970 liters. FWS-B filters and glass fiber filters were both evaluated since glass fiber filters are more commonly used. This report describes the analytical method developed for glass fiber filters.

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

Rhodamine B has been tested in mice and rats by subcutaneous injection and, in inadequate studies, by oral administration. It was carcinogenic in rats when injected subcutaneously, producing local sarcomas. The intravenous LD_{50} in rats is 89.5 mg/kg. (Ref. 5.1)

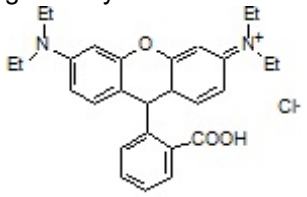
1.1.3 Potential workplace exposure

The following information is taken from the IRAC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. (Ref. 5.1)

Rhodamine B can be used to dye silk, cotton, wool, bast fibres, nylon, acetate fibers, paper, spirit inks and lacquers, soap, wood stains, feathers, leather and distempers on china clay. In the U. S., it has been used as a drug and cosmetic colour in aqueous drug solutions, tablets, capsules, toothpaste, soap, hair-waving fluids, bath salts, lipsticks and rouges. This color has also been used as a tracing agent in water pollution studies, as a dye for waxes and antifreeze and as an analytical reagent for antimony, bismuth, cobalt, niobium, gold, manganese, mercury, molybdenum, tantalum, thallium and tungsten.

No estimate of worker exposure to rhodamine B could be found.

1.1.4 Physical properties (Ref. 5.1 to 5.3)

Molecular weight:	479
Molecular formula:	$C_{28}H_{31}ClN_2O_3$
CAS number:	81-88-9
IMIS number:	0848
Melting point:	165 °C
Solubility:	very soluble in water and alcohol; slightly soluble in hydrochloric acid and sodium hydroxide
Chemical name:	N-[9-(2-carboxyphenyl)-6-(diethylamino)-3H-xanthen-3-ylidene]-N-ethylethanaminium chloride
Synonyms:	tetraethylrhodamine; D & C Red No. 19; rhodamine B chloride; C.I. Basic Violet 10; C.I. 45170
Description:	green crystals or reddish-violet powder
Structure:	

UV data: 546 nm maximum (Ref. 5.1)
556 nm maximum

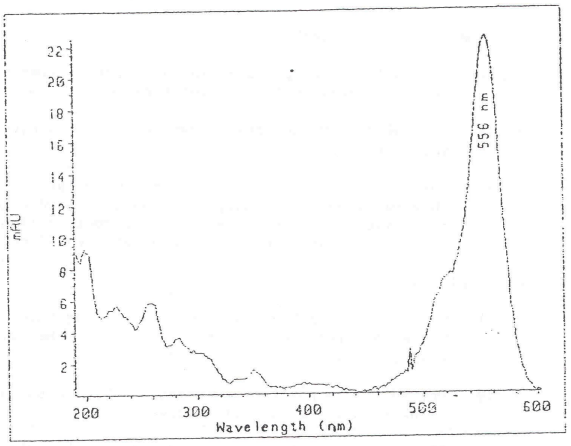


Figure 1.
UV Scan of Rhodamine B in Mobile Phase

1.2 Limit defining parameters

The detection limit of the analytical procedure is 0.013 ng per injection with a fluorescence detector or 0.42 ng per injection with a UV detector. This is the amount of analyte which will give a peak whose height is approximately five times the baseline noise. (Figure 2)

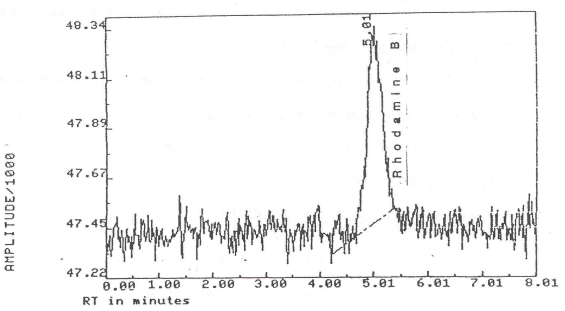


Figure 2.
Detection Limit Chromatogram of Rhodamine B on a Fluorescence Detector

2. Sampling procedure

2.1 Apparatus

2.1.1 A personal sampling pump that can be calibrated to within ± 5% of the recommended flow rate with the sampling device in line.

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

2.1.3 Cassette filter holders for 37-mm filters, Millipore M000037A0 or equivalent.

2.2 Reagents

No sampling reagents are required.

2.3 Sampling technique

2.3.1 Immediately before sampling, remove the plastic plugs from the cassette.

2.3.2 Attach the cassette to the sampling pump with flexible tubing.

2.3.3 Attach the cassette vertically in the employee's breathing zone in such a manner that it does not impede work performance.

2.3.4 After sampling for the appropriate time, remove the cassette and replace the plastic plugs.

2.3.5 Wrap each cassette end-to-end with an OSHA seal (Form 21).

2.3.6 Record the air volume for each sample, and list any possible interferences.

2.3.7 Submit at least one blank for each set of samples. Handle the blank in the same manner as the samples, except no air is drawn through it.

2.3.8 Submit bulk samples for analysis in a separate container. Do not ship them with air samples.

2.4 Extraction efficiency

Six glass fiber filters were each liquid spiked with 6 µL of a 2.21 mg/mL rhodamine B standard. After drying, these filters were each extracted with 5.0 mL of methanol, shaken for 30 min and then analyzed as in Section 3. The results are listed in Table 2.4.

Table 2.4
Desorption Efficiency

amount spiked, µg	amount found, µg	% recovered
13.26	14.24	107.4
13.26	13.47	101.6
13.26	13.26	100.0
13.26	13.19	99.5
13.26	13.28	100.2
13.26	13.42	101.2
	$\bar{x}$	101.6

2.5 Retention efficiency

Eighteen glass fiber filters were each liquid spiked with 6 µL of a 2.21 mg/mL rhodamine B standard. These filters were dried and then 240 L of humid air (~80% relative humidity) were drawn though each filter at approximately 1.3 L/min. Six of the filters were then each extracted with 5.0 mL of methanol, shaken for 30 min and analyzed as in Section 3. The results are listed in Table 2.5. The rest of the filters were kept, 6 in a drawer at ambient temperature and 6 in a refrigerator, for storage studies.

Table 2.5
Retention Efficiency

amount spiked, µg	amount found, µg	% recovered
13.26	12.23	92.2
13.26	12.59	94.9
13.26	11.88	89.6
13.26	12.33	93.0
13.26	11.58	87.3
13.26	11.37	85.7
	$\bar{x}$	90.4

2.6 Sample storage

After 2 days of storage 6 samples, 3 from ambient storage and 3 from refrigerator storage, were each extracted with 5.0 mL of methanol, shaken for 30 min and then analyzed as in Section 3. After 7 days of storage, the remaining samples were extracted and analyzed. The results are given in Tables 2.6.1 and 2.6.2.

Table 2.6.1
Ambient Storage

days stored	amount spiked, µg	amount found, µg	% recovered
2	13.26	10.71	80.8
2	13.26	11.14	84.0
2	13.26	10.68	80.5
7	13.26	12.38	93.3
7	13.26	12.06	91.0
7	13.26	11.64	87.7
		$\bar{x}$ of 2	81.8
		$\bar{x}$ of 7	90.7

Table 2.6.2
Refrigerator Storage

days stored	amount spiked, µg	amount found, µg	% recovered
2	13.26	11.89	89.6
2	13.26	11.91	89.8
2	13.26	12.13	91.4
7	13.26	11.44	86.3
7	13.26	11.89	89.7
7	13.26	12.07	91.0
		$\bar{x}$ of 2	90.3
		$\bar{x}$ of 7	89.0

2.7 Recommended air volume and sampling rate

2.7.1 The recommended air volume is 240 L.

2.7.2 The recommended flow rate is 1.0 L/min.

2.8 Interferences (sampling)

It is not known if any compounds will interfere with the collection of rhodamine B. Any suspected interferences should be reported to the laboratory.

2.9 Safety precautions (sampling)

2.9.1 Attach the cassette in such a manner that it will not impede work performance or employee safety.

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

3. Analytical procedure

3.1 Apparatus

3.1.1 A balance capable of weighing to the nearest tenth of a milligram. A Mettler HL52 balance was used in this evaluation.

3.1.2 A mechanical shaker.

3.1.3 An HPLC with UV and fluorescence detectors. A Hewlett Packard 1090 liquid chromatograph with a diode array detector and an ABI Analytical (980G) fluorescence detector were used in this evaluation.

3.1.4 An HPLC column capable of separating rhodamine-B from any interferences. A 100 mm × 2.1 mm i.d. Hypersil ODS, 5 µm, column was used in this evaluation.

3.1.5 An electronic integrator or some other suitable means for measuring detector response. The Hewlett-Packard (HP) 1090 Chem Station and HP 3357 data system were used in this evaluation.

3.1.6 Volumetric flasks and pipets of various sizes.

3.1.7 Scintillation vials, 20-mL.

3.1.8 Vials, 2-mL.

3.2 Reagents

3.2.1 Methanol, HPLC grade.

3.2.2 Water, HPLC grade.

3.2.3 Acetonitrile, HPLC grade.

3.2.4 Phosphoric acid (H_3PO_4), reagent grade.

3.2.5 Rhodamine B. An Aldrich standard of 99% purity was used in this evaluation.

3.3 Standard preparation

Prepare rhodamine B stock standards by weighing 10 to 15 mg of rhodamine B. Transfer the rhodamine B to separate 10-mL volumetric flasks and add methanol to the mark. Make working range standards of 0.01 to 5.0 µg/mL by pipet dilutions of the stock standards with methanol. Store stock and dilute standards in a freezer.

3.4 Sample preparation

3.4.1 Transfer the glass fiber filter to a scintillation vial.

3.4.2 Add 5.0 mL of methanol to each vial and seal with a Teflon-lined cap.

3.4.3 Shake the vials for 30 minutes on a mechanical shaker.

3.4.4 Transfer the sample to a 2-mL vial for use in an HP autosampler.

3.5 Analysis

3.5.1 Instrument conditions

Column:	100 mm × 2.1 mm i.d. Hypersil ODS, 5 µm
Oven temperature:	40 °C
Mobile phase:	85% acetonitrile 15% water with 0.005M 1-heptanesulfonic acid and the pH adjusted to 3.5 with H_3PO_4
Flow:	0.2 mL/min
Wavelength:	556 nm
Fluorescence detector:	Excitation: 210 nm

Emission: 550 nm cut off filter
Injection volume: 1.0 µL
Retention time: 4.5 min

3.5.2 Chromatogram:

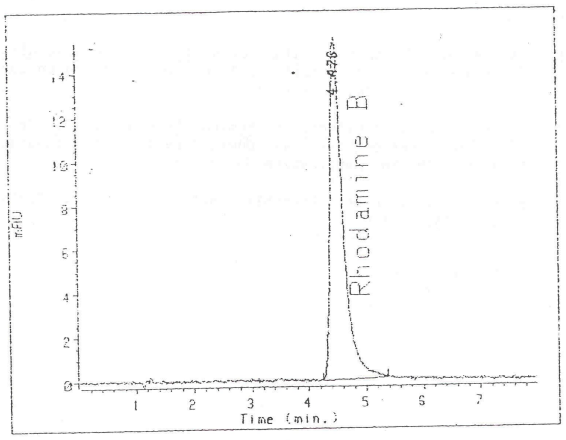


Figure 3.
Chromatogram of Rhodamine B on a UV Detector

3.6 Interferences (analytical)

3.6.1 Any collected compound having a retention time similar to that of rhodamine B is an interference.

3.6.2 Generally, HPLC conditions may be varied to circumvent interferences.

3.6.3 Retention time on a single column is not proof of chemical identity. Analysis by an alternate HPLC column, comparison of detector responses or confirmation by mass spectrometry are additional means of identification.

3.7 Calculations

3.7.1 Construct a calibration curve by plotting detector response versus concentration (µg/mL) of rhodamine B.

3.7.2 Determine the µg/mL of rhodamine B in each sample and blank from the calibration curve.

3.7.3 Blank correct, if necessary, the sample by subtracting the µg/mL found in the blank from the µg/mL found in the sample.

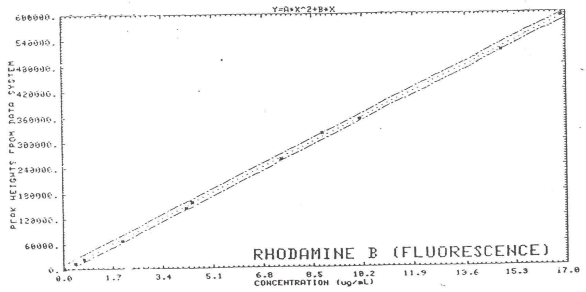


Figure 4.
Calibration Curve

3.7.4 Determine the air concentration by using the following formula.

$$mg/m^3 = \frac{(mg/mL \text{ blank corrected})(\text{extraction volume, mL})}{(\text{air volume, L})(\text{extraction efficiency, decimal})}$$

3.8 Safety precautions (analytical)

3.8.1 Avoid skin contact and air exposure to rhodamine B.

3.8.2 Avoid skin contact with all solvents.

3.8.3 Wear safety glasses at all times.

4. Recommendation for further study

This method should be fully validated.

5. References

5.1 IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man; International Agency for Research on Cancer: Lyon, 1978; Vol. 16, pp 221-231.

5.2 Registry of Toxic Effects of Chemical Substances 1985-86 Edition; U.S. Department of Health and Human Services: Cincinnati, OH, 1987; DHHS(NIOSH) Publication No. 87-114, p 313.

5.3 Merck Index, 10th ed.; Windholz, Martha Ed.; Merck: Rathway, N.J., 1983; p 1180.