TECHNICAL REPORT 9112

DETERMINATION OF SELECTED COLORED SMOKES ON GLASS FIBER DISCS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

ERNST E. BRUEGGEMANN
ALAN B. ROSENCRANCE
WILLIAM E. DENNIS

U S ARMY BIOMEDICAL RESEARCH & DEVELOPMENT LABORATORY
Fort Detrick
Frederick, MD 21701-5010

May 1991

U S ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
Fort Detrick
Frederick, MD 21701-5012

8c. ADDRESS (City, State, and ZIP Code)
Building 568, Fort Detrick
Frederick, MD 21702-5010

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification) (U) Determination of Selected Colored Smokes on Glass Fiber Filter Discs by High Performance Liquid Chromatography (HPLC)

12. PERSONAL AUTHOR(S)
Ernst F. Brueggemann, Alan B. Rosencrance, and William E. Dennis

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Technical	FROM 6/89 TO 3/90	1991 June 3	12

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
High Performance Liquid Chromatography (HPLC), Analytical Method, 1,4-diamino-2,3-dihydroanthraquinone, 2-(2'-quinolinyl)-1,3-indandione, colored smokes, 1-methylamino-

19. ABSTRACT (Continue on reverse if necessary and identify by block number)
Liquid chromatographic method has been developed for the determination of 1,4-diamino-2,3-dihydroanthraquinone (DDA), 2-(2'-quinolinyl)-1,3-indandione (QID), 1-methylamino-anthraquinone (MAA), and 1,4-di-p-toluidinoanthraquinone (DTA).

A relatively rapid and reliable HPLC method has been developed for the determination of selected colored smokes on glass fiber filter discs. The sample preparation process was streamlined by extracting the colored-smokes/disc samples into acetonitrile followed by filtration and direct injection into a high performance liquid chromatograph equipped with a programmable ultraviolet (UV) spectrophotometric detector. A mixed-mode C18/cation column provided adequate resolution and optimal peak symmetry for all four of the colored smokes investigated. The precision and accuracy data and detection limits are included in the report.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Alan B. Rosencrance	(301) 663-2340	SGRD-UBG-R

18. Continued: anthraquinone (MAA), and 1,4-di-p-toluidinoanthraquinone (DTA).

NOTICE

Disclaimers

The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Research was conducted in compliance with the Animal Welfare Act, and other Federal statues and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 86-23, 1985 edition.

Disposition

Destroy this report when it is no longer needed. Do not return it to the originator.

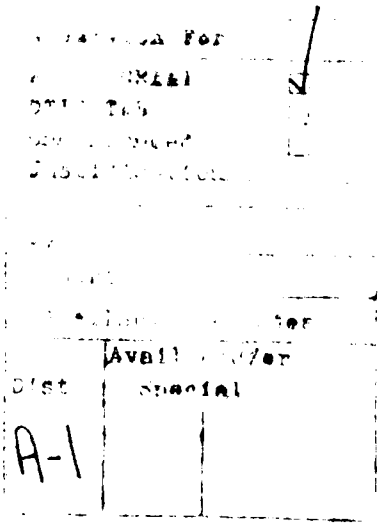


TABLE OF CONTENTS

TABLES

FIGURES

INTRODUCTION AND OBJECTIVES

The U.S. Army Biomedical Research and Development Laboratory has been involved in studies to determine the extent of a soldier's exposure to toxic substances generated by the normal use of military weapon/systems. One of the contaminants routinely encountered in military training is colored signaling smokes from M18 grenades. These grenades contain one or more of the following compounds as a coloring agent: 1,4-diamino-2,3-dihydroanthraquinone (DDA), 2-(2'-quinolinyl)-1,3-indandione (QID), 1-methylamino-anthraquinone (MAA), and 1,4-di-p-toluidinoanthraquinone (DTA).

Concern for exposure of workers, instructional cadre, and trainees during military training exercises has increased due to mounting evidence of the bacterial mutagenic activity of some anthraquinone related colored smokes[1] and QID[2]. This study was part of a larger project to determine the actual exposures of these four compounds received by soldiers during training exercises. This work's object was to develop a method to detect these compounds in atmospheric samples collected during training exercises.

Current analytical techniques employed for the separation and analysis of colored smokes are thin layer chromatography (TLC)[3-5] and gas chromatography (GC)[6]. Thin layer chromatography provides simple detection of colored smokes; however, quantitation suffers from the crudeness inherent in most TLC analyses. Gas chromatography has been successfully applied to some anthraquinone dyes and intermediates, but has limited application to dyes in general because these compounds have high molecular weights, low vapor pressures and low thermal stability.

High performance liquid chromatography (HPLC) appears to be the analytical method of choice since HPLC can be conducted at ambient temperatures without loss in resolution or speed of analysis. Also, HPLC has been proven to be suitably accurate and precise in a wide range of dye applications[7-9].

The work herein describes a rapid, reliable, and sensitive HPLC method for the determination of colored smokes in atmospheric samples.

METHODS AND MATERIALS

CHROMATOGRAPHIC SYSTEM

A Waters liquid chromatographic system (Waters Chromatography Division, Milford, MA) was used throughout the study. The system consisted of the following components: two model 6000A solvent delivery systems, a model 721 programmable system controller, a model 730 data module, and a model 710B WISP autosampler. The UV detector was a Spectroflow 783 programmable absorbance detector (Applied Biosystems, Inc., Ramsey, NJ) set at 300 nm (0.005 aufs). An IBM octadecyl column (25 cm x 4.6 mm i.d., 5 micron particle size, IBM Instruments, Inc., Danbury, CT) and a Alltech Mixed-Mode RP-C18/Cation column (25 cm x 4.6 mm i.d., 7 micron particle size, Alltech Assoc., Inc., Deerfield, IL) were used for the separation of the dye compounds. A linear gradient elution program was used in which the eluent was changed from 100 percent 0.02 M potassium phosphate, monobasic (pH 4.5) to 100 percent acetonitrile/water (90:10) in 30 minutes at 2 mL/min. The injection volume was 25 microliters.

REAGENTS AND MATERIALS

HPLC grade acetonitrile was obtained (Baxter Healthcare Corp., Burdick and Jackson Division, Muskegon, MI) and was used without further purification. Potassium phosphate (A.C.S. certified, monobasic) was purchased from Fisher Scientific Co., Fair Lawn, NJ. Water for HPLC was purified with a Milli-Q™ water purification system (Millipore Corp., Bedford, MA). Glass fiber filters (Nucleopore Corp., Pleasanton, CA) were used for collection of dyes from the air. Centrex centrifugal microfilters (0.5 micron, Scheicher and Schell, Inc., Keene, NH) were purchased for the filtration of glass fiber filter extracts.

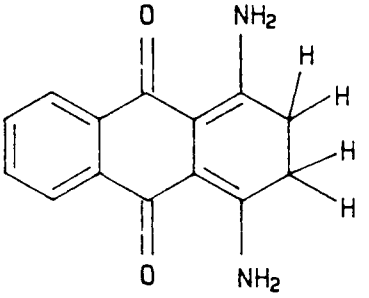
The standards of 1-(methylamino)anthraquinone, 1,4-di-p-toluidinoanthraquinone and 2-(2'-quinolyl)-1,3-indandione were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI.

Analytical grade 1,4-diamino-2,3-dihydroanthraquinone was obtained from Pfaltz and Bauer, Inc., Waterbury, CT.

The structural formula and other pertinent data for each of the four dyes are as follows:

DDA

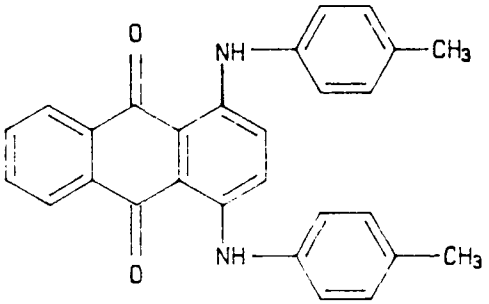
CAS Registry Number: 81-63-0
M.W.: 240.26
Chemical Formula: C14-H12-O2-N2
Structure:



Synonyms: DDA; 1,4-Diamino-2,3-dihydro-9,10-anthracenedione; 1,4-Diamino-2,3-dihydroanthraquinone; Solvent Violet 47[10].

DTA

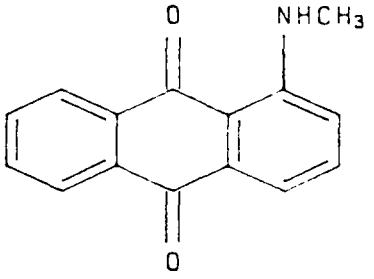
CAS Registry Number: 128-80-3
RETCS Reference Number: CB5775000
Color Index (C.I.) Number: 61565
M.W.: 418.52
Chemical Formula: C28-H22-N2-O2
Structure:



Synonyms: C.I. Solvent Green 3; Cyanine Green Base 3; Alizarine Cyanine Green Base; Alizarine Green G Base; Amaplast Green OZ; 1,4-Bis((4-methylphenyl)amino)-9,10-Anthracenedione (9CL); Anthraquinone Green G Base; Arlosol Green B; Arlosol Green BS; Arlosol Green BBS; Bis-1,4-p-tolyaminoathrchion (CZECH); C-Green 10; C.I. 61565; D and C Green No.6; Fat Soluable Green Anthraquinone; 11091 Green; Green No. 202; Micro-lex Green 5B; Nitro Fast Green GB; Organol Fast Green J; Organol Green J; Quinazarin Green; Quinizarine Green Base; Quinizarin Green SS; Solvent Green 3; Sudan Green 3; Sudan Green 4B; Toyo Oriental Oil Blue G; Waxoline Green; Waxoline Green G[10].

MAA

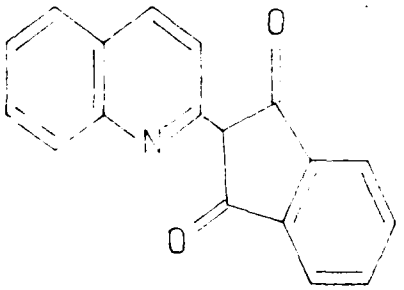
CAS Registry Number: 82-38-2
Color Index Number: 60505
M.W.: 237.26
Chemical Formula: C15-H11-N-O2
Structure:



Synonyms and Trade Names: Disperse Red 9; 1-(methylamino)-9,10-anthracenedione: 1-(methylamino)-anthraquinone; Solvent Red 111: Amaplast Red AAP; Calco Oil Red ZMQ; Duranol Red GN; Macro-lex Red G; Orient Oil Red 330[10].

QID

CAS Registry number: 8003-22-3
RETCS Reference Number: GE5925000
Color Index Number: 47000
M.W.: 273.30
Chemical Formula: C18-H11-N-O2
Structure:



Synonyms and Trade Names: Solvent Yellow 33; Arlosol Yellow S; Chinoline Yellow D Sol. in Spirits; Chinoline Yellow ZSS; CI 47000; D and C Yellow No. 11; Nitro Fast Yellow SL; Oil Yellow SIS; Petrol Yellow C; Quinoline Yellow A Spirit Soluble; Quinoline Yellow Base; Quinoline Yellow Spirit Soluble; Quinoline Yellow SS; Solvant Yellow 33; Waxoline Yellow T[10].

SAMPLE PREPARATION

Air particulate samples were obtained by drawing a known volume of air through a glass fiber filter. Each glass fiber filter was then placed in a 10 mL glass screw-cap test tube containing 3 mL of acetonitrile. The contents of each tube were vortexed vigorously for 2 minutes and then clarified by centrifugation through a 0.5 micron centrifugal microfilter.

PREPARATION OF STOCK AND STANDARDS

A stock solution containing the dyes of interest was prepared by dissolving 10 mg of each compound in 100 mL of acetonitrile (using ultrasonication) to yield a concentration of 100 mg/L. Fresh working standards were prepared by dilution of the stock solution with acetonitrile to furnish standards with concentrations of 0.2, 1.0, 2.0, and 5.0 mg/L respectively. The stock and standard solutions are made fresh on the day of analysis.

CALCULATIONS

Peak areas for all working standards were plotted against their concentrations to obtain a standard curve. The peak area of the sample unknown was compared to the appropriate standard curve to obtain a concentration in mg/L. Next, the sample'sconcentration (mg/L) obtained from the standard curve was converted to μg/3 mL to obtain a concentration in μg/glass fiber filter.

RESULTS AND DISCUSSION

Figures 1a and 1b show the chromatographic separation and detection of the four dye standards using a mixed-mode C18/cation and a C18 column, respectively. The mobile phase for both figures is the same and is listed in the experimental section.

In Figures 1a and 1b, both columns provide adequate resolution and peak symmetry for QID, MAA, and DTA. However, the C18 column used in Figure 1b could not provide the needed peak symmetry and sensitivity needed for DDA. DDA's poor peak symmetry and band tailing could possibly be explained by the fact that some basic solutes can interact with the residual silanols present on the C18 column causing chromatographic band tailing[11].

Figure 1a shows a dramatic improvement in peak symmetry and sensitivity to DDA. The column used in Figure 1a (mixed-mode C18/cation) contains a spherical silica substrate that has been bonded with carboxylate functionalities in addition to C18 functionalities[12]. When the pH of the mobile phase is above 4, the ionization of the carboxylate function increases and can serve as a cation exchanger for basic solutes.

Figures 2a and 2b show an HPLC chromatogram of an acetonitrile extract of a glass fiber filter blank and an acetonitrile extract of a glass fiber filter spiked with the appropriate colored smokes, respectively. The chromatogram in

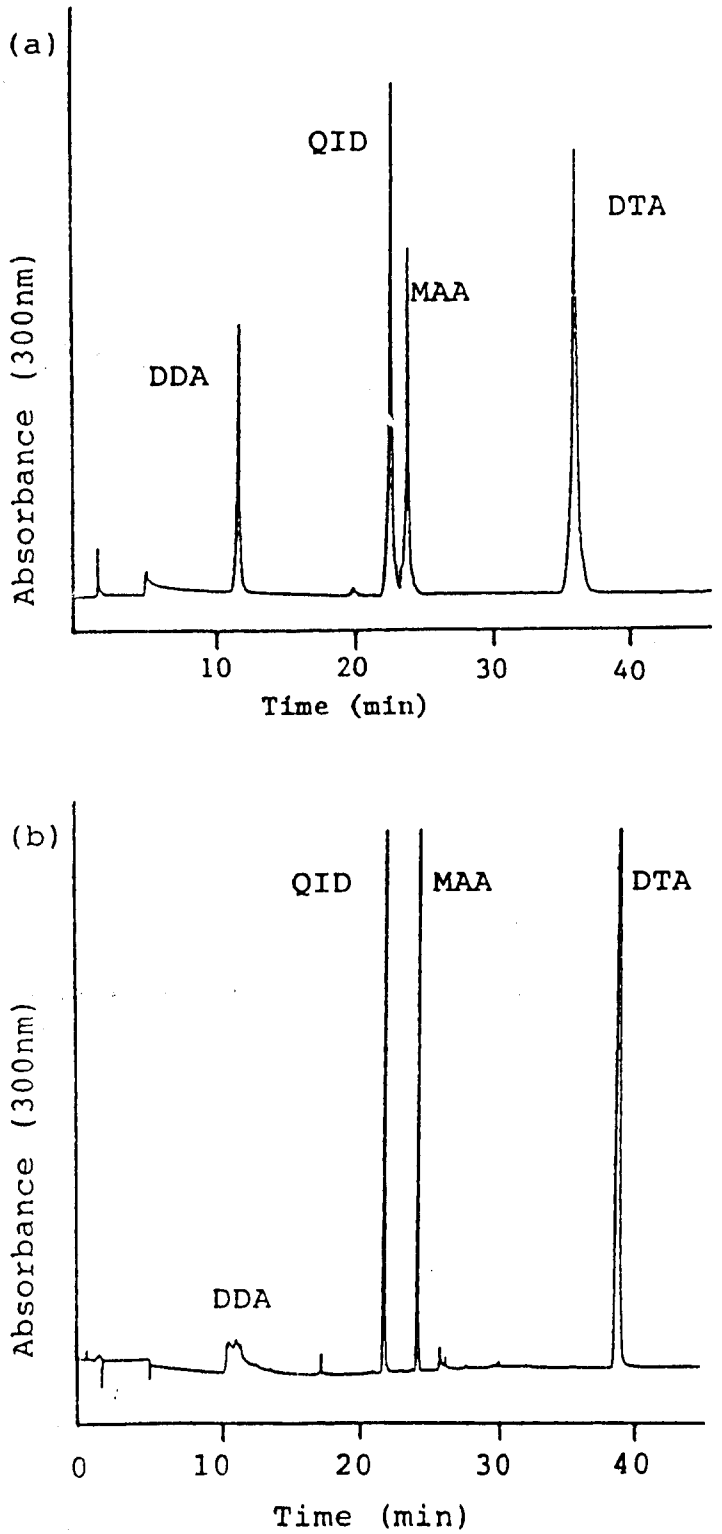


Figure 1. HPLC chromatograms of colored smoke standards using (a) a mixed-mode C18/cation column and (b) a C18 column. Mobile Phase: linear gradient (30 min A->B), A=0.02M KH_2PO_4 buffer (pH 4.5), B= acetonitrile/water (90:10), Flow Rate: 2mL/min, Detector: 300 nm UV.

Figure 2a (blank) showed the absence of any chromatographic peaks that might interfere with the colored smoke peaks shown in Figure 2b.

The determination of detection limits for each compound closely parallels the criteria set forth by Hubaux and Vos[13]. The mean peak area for each working standard was plotted against its concentration to obtain a standard curve. Next, spiked glass fiber filter extracts were prepared and their concentrations were determined by regression analysis using the appropriate standard curve. The determined value found concentrations for each colored smoke was plotted against its known concentration (Figure 3). The colored smoke DTA was used here as an example. The regression line was prepared for each colored smoke by plotting the found concentration (Y-axis) versus its known concentration (X-axis). The upper and lower confidence limits (95 percent) were established along the regression line. The detection limit was estimated by drawing a horizontal line from the upper confidence limit curve to the lower confidence limit curve, at which point the line was extrapolated vertically to a corresponding known concentration. This is the level of colored smoke that can be detected and distinguished from zero with a 95 percent confidence. The detection limit for each colored smoke is listed in Table 1.

Table 1. Detection Limits (μg/Filter) for Colored Smokes on Glass Fiber Filters.

COMPOUND	DETECTION LIMIT
DDA	0.72
QID	0.60
MAA	0.90
DTA	0.70

The amount recovered, standard deviation, relative standard deviation and percent recovery for glass fiber filters spiked with the appropriate colored smoke is given in Table 2. Low level recoveries ranged from 88.6 percent for DDA to 99.6 percent for DTA. High level recoveries ranged from 95.1 percent for QID to 106.9 percent for DDA. The relative standard deviation for colored smoke determinations was less than 4.00 percent at both low and high concentration levels.

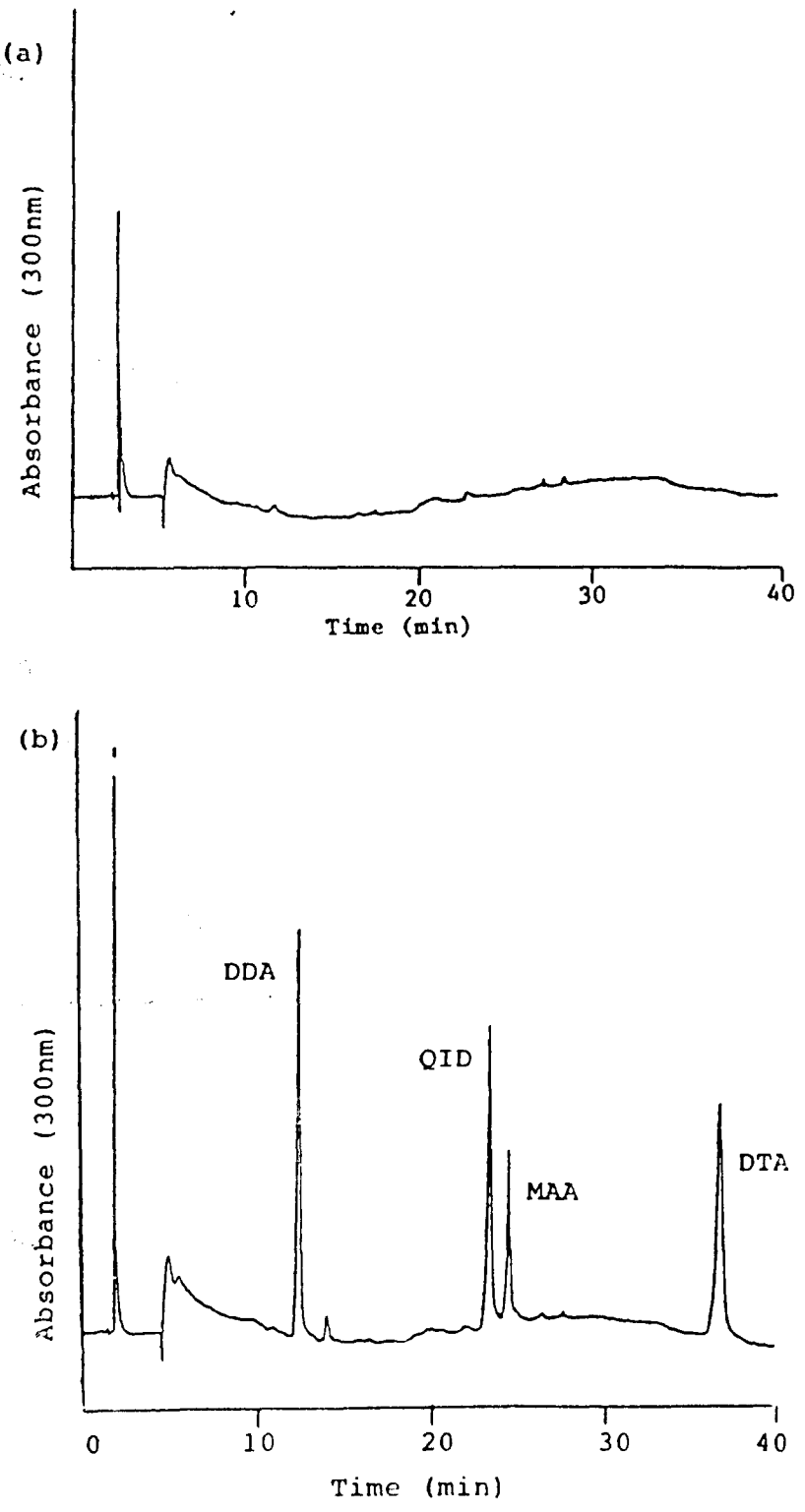


Figure 2. HPLC chromatogram of (a) an extract of a glass fiber filter blank and (b) a glass fiber filter spiked with the appropriate colored smokes. Mobile Phase: linear gradient (30 min. A->B), A=0.02M KH_2PO_4 buffer (pH 4.5), B= acetonitrile/water (90:10), Flow Rate: 2 mL/min, Detector: 300 nm UV.

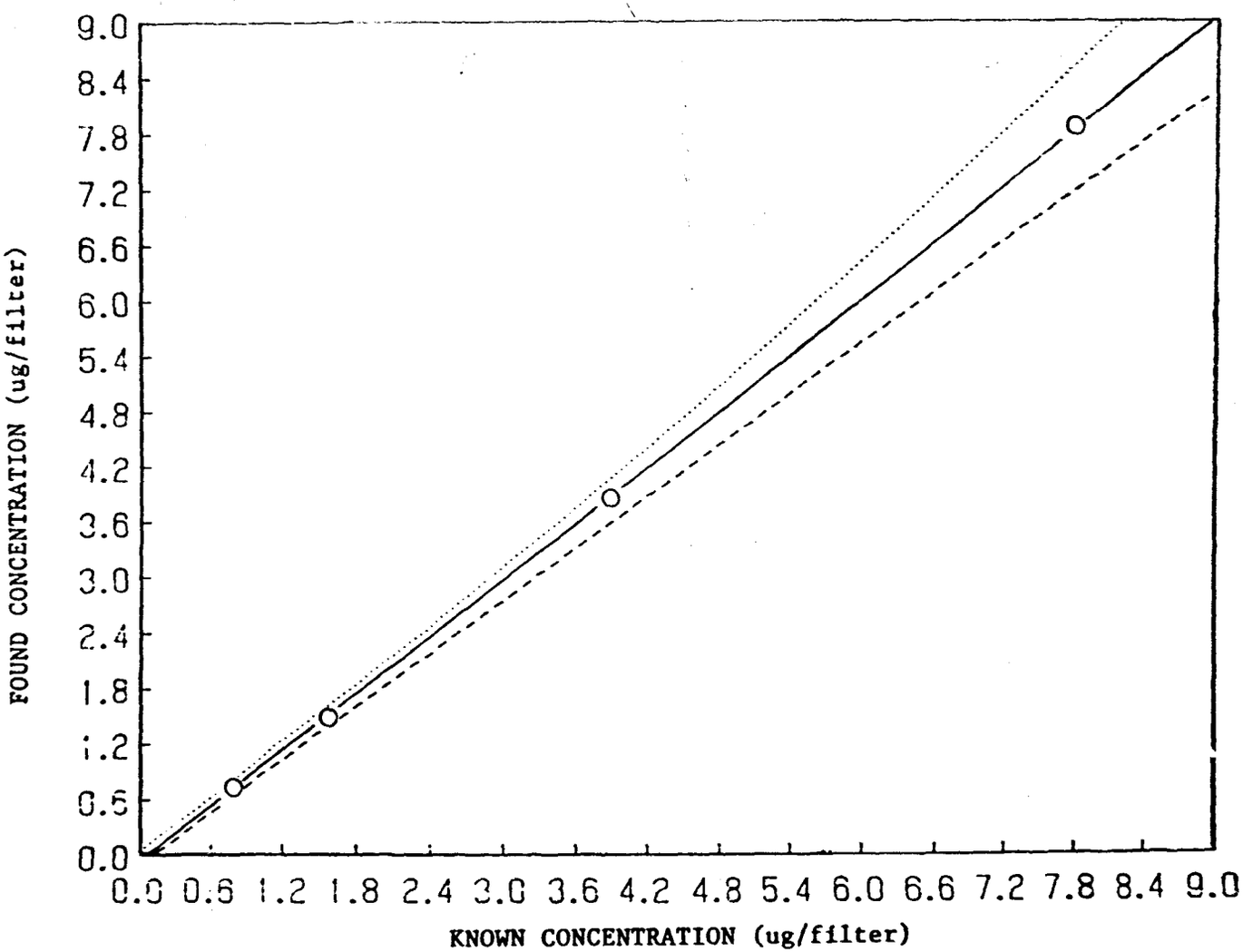


Figure 3. Calibration plot for the determination of DTA detection limit.

Table 2. Accuracy of Colored Smoke Determination on Glass Fiber Filters

Compound	Amount Added (μg/Filter)	Amount[a] Recovered (μg/Filter)	S.D.	R.S.D. (%)	REC. (%)
		LOW LEVEL			
DDA	2.19	1.94	0.06	3.04	88.6
QID	2.07	1.89	0.03	1.69	91.3
MAA	2.14	2.00	0.07	3.55	93.5
DTA	2.24	2.23	0.06	2.74	99.6
		HIGH LEVEL			
DDA	8.76	9.36	0.24	2.61	106.9
QID	8.31	7.90	0.04	0.49	95.1
MAA	8.54	8.15	0.11	1.36	95.4
DTA	8.98	8.74	0.06	0.69	97.3

[a] Mean of three determinations.

CONCLUSION

A relatively rapid and reliable HPLC method has been developed for the determination of selected colored smokes on glass fiber filter discs.

The sample preparation process was streamlined by merely extracting the colored-smokes/disc sample into acetonitrile followed by filtration and direct injection onto a high performance liquid chromatograph (HPLC).

A mixed-mode C18/cation column provided adequate resolution and optimal peak symmetry for all four of the colored smokes investigated. In addition, monitoring the column effluent spectrophotometrically at 300 nm allowed for good sensitivity of the colored-smokes while minimizing background interferences.

LITERATURE CITED

1. Owens E.J. and D.M. Ward. 1974. A review of the toxicology of colored chemical smokes and colored smoke dyes. Technical Report EB-TR-74064. Biomedical Laboratory, Edgewood Arsenal, Aberdeen Proving Ground, MD.

2. M. Moore, J. Allen, and L. Claxton. 1984. Mutagenic screening of three dyes for marker grenades in the Salmonella reversion assay, the L5178Y/TK+/- mouse lymphoma assay, and In Vivo sister chromatid exchange in mice. Final Report, AD A152110. U.S. Environmental Protection Agency, Research Triangle Park, NC.

3. J.C. Brown. 1969. Chromatography and identification of dyes. J. Soc. Dyers Colourists. 85(4):137-46.

4. G. Zweig and J. Sherma. 1972. Chromatography. Anal. Chem. 44(5):42R-79R.

5. A.M. Arsov, B.K. Mesrob and A.B. Gateva. 1973. Thin-layer chromatography of basic dyes. J. Chromatogr. 81:181-186.

6. J.B. Terrill and E.S. Jacobs. 1970. Application of gas-liquid chromatography to the analysis of anthraquinone dyes and intermediates. J. Chromatogr. Sci. 8:604-607.

7. R.P. Scott and C.E. Reese. 1977 Precision of contemporary liquid chromatographic measurements. J. Chromatog. 138:283-307.

8. Fisher, D.T., D.T. Burton, M.J. Lenkevich and K.R. Cooper. 1985. Analytical methods for determining the concentration of solvent yellow 33, solvent green 3, synthetic-HC smoke combustion products and DEGDN in freshwater aquatic organisms. Final Report for Phase I, AD A167875. The Johns Hopkins University, Applied Physics Laboratory, Laurel, MD. NAVSEA N00024-85-C-5301 via Army MIPR85MM5505.

9. Gosnell, A.B. 1976. Methods for analysis of contaminated soil: Pine Bluff Arsenal, Pine Bluff, Arkansas. Contract DAAA-75-C-0183. Department of the Army Headquarters, Edgewood Arsenal, Aberdeen Proving Ground, MD.

10. Registry of Toxic Effects of Chemical Substances. 1985. U.S. Department of Health, Education and Welfare Public Health Service, Center of Disease Control, National Institute for Occupational Safety and Health. Vol. I and II.

11. L.R. Snyder, J.L. Glajch and J.J. Kirkland. 1988. Pratical HPLC Method Development. John Wiley and Sons, Inc., New York.

12. H.J. Issac and J. Gutierrez. 1988. Mixed packings in high performance liquid chromatography: II. Mixed packings vs. mixed ligands. J. Liquid Chromatogr., 11(14), 2851-2861.

13. A. Hubaux and G. Vos. 1970. Decision and detection limits for linear calibration curves. Anal. Chem. 42(8):849-855.

DISTRIBUTION LIST

4 Commander
U.S. Army Medical Research and Development Command
ATTN: SGRD-RMI-S
Fort Detrick
Frederick, MD 21702-5012

2 Commander
U.S. Army Biomedical Research and Development Laboratory
ATTN: SGRD-UBZ-IL
Fort Detrick
Frederick, MD 21702-5010

2 Defense Technical Information Center (DTIC)
ATTN: DTIC-FDAC
Cameron Station
Alexandria, VA 22304-6145

1 Commander
U.S. Army Biomedical Research and Development Laboratory
ATTN: SGRD-UBZ-P
Fort Detrick
Frederick, MD 21702-5010

2 Commandant
Academy of Health Sciences, U.S. Army
ATTN: HSHA-DCD
Fort Sam Houston, TX 78234-6100