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STANDARD OPERATING PROCEDURES AND TABLE OF CONTENTS FOR APPENDIX E OF
THE DRAFT CORRECTIVE MEASURES IMPLEMENTATION PLAN/QUALITY ASSURANCE
PROJECT PLAN SOLID WASTE MANAGEMENT UNIT 2 (SWMU 2) WITH TRANSMITTAL
NSA CRANE IN
6/12/2006
NSA CRANE



DEPARTMENT OF THE NAVY

CRANE DIVISION
 NAVAL SURFACE WARFARE CENTER
 300 HIGHWAY 361
 CRANE INDIANA 47522-5001

IN REPLY REFER TO:

5090/S4.7.7
 Ser PRCR4/6179
 12 JUN 2006

U.S. Environmental Protection Agency, Region V
 Waste, Pesticides, & Toxics Division
 Waste Management Branch
 Corrective Action Section
 77 West Jackson Blvd.
 Chicago, IL 60604

Dear Mr. Ramanauskas:

Crane Division, Naval Surface Warfare Center submits the Standard Operating Procedure (SOP) and Table of Contents (TOC) for Appendix E of the Draft Corrective Measures Implementation Plan/Quality Assurance Project Plan (CMIP/QAPP) for the Dye Burial Grounds (DBG), Solid Waste Management Unit 02. One copy is submitted for inclusion into the CMIP/QAPP as enclosure (1). The permit required Certification Statement is provided as enclosure (2).

If you require any further information, my point of contact is Mr. Thomas J. Brent, Code PRCR4-TB, at 812-854-6160, email thomas.brent@navy.mil.

Sincerely,

J. M. Hunsicker

J. M. HUNSICKER
 Environmental Site Manager
 By direction of the Commanding Officer

Enclosures: 1. SOP & TOC for Appendix E of the Draft DBG
 CMIP/QAPP
 2. Certification Statement

Copy to:
 ADMINISTRATIVE RECORD
 SOUTHNAVFACENGCOM (Code ES31) (w/o encl)
 IDEM (Doug Griffin)
 TTNUS (Ralph Basinski) (w/o encl)

I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.



SIGNATURE

Manager, Environmental Protection

TITLE

6/12/06

DATE

**APPENDIX E
LABORATORY STANDARD OPERATING PROCEDURES**

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CR4052-PD-2500	Determination of Organic Colorants in Environmental Matrices by High-Performance Liquid Chromatography

**Determination of Organic Colorants in Environmental Matrices by
High-Performance Liquid Chromatography**

Explosive Sciences Branch
Naval Surface Warfare Center Crane
300 Highway 361
Crane, IN 47522

May 9, 2001

Approvals:

Name: Ralph Morris

Title: Branch Head

Signature _____

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1.0 SCOPE AND APPLICABILITY

This method is intended for the analysis of organic colorants by high performance liquid chromatography (HPLC) using a Photodiode Array Detector (PDA). This method is used to determine the concentration of the following dyes in aqueous and solid matrices:

Aqueous Soluble			Organic Soluble		
Compound Name	Abbr.	CAS #	Compound Name	Abbr.	CAS #
Acid Blue 1	AB1	129-17-9	Disperse Blue 14	DB14	2475-44-7
Acid Blue 9	AB9	3844-45-9	Disperse Red 9	DR9	82-38-2
Acid Blue 45	AB45	2861-02-1	Disperse Violet 1	DV1	128-95-0
Acid Orange 10	AO10	1936-15-8	Solvent Green 3	SG3	128-80-3
Acid Red 64	AR64	NA	Solvent Orange 3	SO3	495-54-5
Acid Yellow 3	AY3	8004-92-0	Solvent Orange 7	SO7	3118-97-6
Acid Yellow 23	AY23	1934-21-0	Solvent Red 1	SR1	1229-55-6
Acid Yellow 73	AY73	518-47-8	Solvent Red 24	SR24	85-83-6
Basic Violet 10	BV10	81-88-9	Solvent Yellow 2	SY2	60-11-7
Basic Yellow 2	BY2	2465-27-2	Solvent Yellow 3	SY3	97-56-3
			Solvent Yellow 14	SY14	842-07-9
			Solvent Yellow 33	SY33	8003-22-3
			1-Aminoanthraquinone	1AQ	82-45-1
			2-Aminoanthraquinone	2AQ	117-79-3

- 1.1 This method provides a solid phase extraction procedure for dyes in surface or ground water.
- 1.2 These dyes may be dangerous. When handling samples and stock solutions, follow safety precautions listed in Section 4.0 and 5.0.

2.0 SUMMARY OF THE METHOD

- 2.1 This method provides HPLC conditions for the detection of ppb and low ppm levels of dyes in aqueous and solid matrices. Prior to using this method, appropriate sample preparation must be used.
- 2.2 This method utilizes a dual gradient, where flow and mobile phase composition are varied over time, to achieve sufficient separation of the target analytes.
- 2.3 This method provides two individual gradient methods for the separation of the analytes. The short gradient method (40 minutes total) provides for rapid separation of the analytes with the loss of resolution between two coeluting pairs of analytes (Acid Yellow

3/Acid Yellow 23 and Acid Blue 1/Disperse Violet 1). The long gradient method (70 minutes total) provides for the separation of the two coeluting pairs from the short gradient method. For soils, the long gradient method will only be run if one of the analytes in the coeluting pairs is detected in the short gradient method. The long gradient is used for aqueous samples because the percent recovery is biased high in the short gradient. Therefore, quantification of aqueous samples will be based on the long gradient.

- 2.4 Aqueous Sample Extraction: This method is an adaptation of a membrane solid phase extraction procedure for nonvolatile organics described in EPA Method SW-846-8330. Aqueous samples are extracted using a C18 extraction disk and tetrabutylammonium hydroxide. A small volume of methanol extracts the analytes from the C18 disk and is concentrated by rotary evaporation.
- 2.5 Soil and Sediment Sample Extraction: Solid samples are extracted with a 5:5:50 v/v mixture of tetrabutylammonium hydroxide (in methanol): water:methanol and filtered prior to analysis.
- 2.6 Acid Blue 45 exhibits significantly reduced recoveries in particulate-contaminated aqueous matrices. This is due to the preferential binding of Acid Blue 45 to particulates because of its high polarity.
- 2.7 Basic Yellow 2 results are biased high in both solid and aqueous matrices. While this method can confirm the presence of basic yellow 2 in a sample matrix, the quantitative results will be significantly higher than the actual amount in the matrix.
- 2.8 Acid Yellow 23 deviates significantly in ICV and CCV despite considerable effort to correct the problem. Therefore, Acid Yellow 23 is exempt from ICV and CCV criteria and corrective action.

3.0 DEFINITIONS

This section defines the terms and acronyms as used in this SOP.

PDA	Photodiode Array Detector
CCV	Continuing Calibration Verification - Standard that is injected at some prescribed frequency during the analytical run sequence to determine if the instrument is still calibrated.

ICV	Initial Calibration Verification – Standards that are injected at the beginning of a 24 analytical run sequence to determine if the instrument is still calibrated.
LC	Liquid Chromatography
RT	Retention Time - The time (in minutes) at which a target analyte elutes from the LC column.
RT Window	Retention Time Window - The +/- value which is applied to the ICV to establish the time range used to make tentative compound identifications.
UV/VIS	Ultraviolet/Visible absorption spectra

4.0 HEALTH AND SAFETY WARNINGS

- 4.1 Standard laboratory protective clothing and eye covering is required during the execution of this method.

5.0 CAUTIONS

- 5.1 Refer to the instrument manufacturer's manual for routine instrument precautions. This includes an awareness of moving parts and the potential for electrical shock.
- 5.2 Waste Disposal – All waste will be disposed of according to federal, state, and local regulations.
- 5.3 Routine and Preventative Maintenance

PREVENTIVE MAINTENANCE FOR ANALYTICAL INSTRUMENTS

Instrument	Preventive Maintenance	Maintenance Frequency
HPLC	Change filter frit in mixer. Change column pre-filter. Rinse water pump with methanol, filter water, sonicate water intake filter frit. Change pump seals.	As needed (when pressure builds) As needed (2-3 months) Approximately weekly. As needed.

6.0 INTERFERENCES

- 6.1 Solvent, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences.
- 6.2 Several dye compounds decompose rapidly when exposed to light. Standards must be kept in a dark cool area until used. Samples and sample extracts must be stored in a dark cool area until analyzed.

7.0 PERSONNEL QUALIFICATIONS

This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC, skilled in the interpretation of chromatograms, and experienced in handling dye materials. Each analyst must demonstrate the ability to generate acceptable results with this method.

8.0 APPARATUS AND MATERIALS

8.1 HPLC System

- Waters 2690 Separations Module (auto-sampler, gradient pump)
- Waters 996 Photodiode Array Detector
- Waters Temperature Control Module
- 30 uL minimum volume sample loop
- Waters Millennium³² Chromatography Software

The system is equipped with a Waters 2690 Separation Module. The pump is capable of achieving 5000 psi. For the low concentration option, the detector must be capable of a stable baseline at 0.001 absorbance units full scale.

8.2 Other Equipment

8.2.1 Temperature controlled ultrasonic bath

8.2.2 Balance ± 0.0001 g

8.2.3 Oven

8.3 Materials

8.3.1 Disposable cartridge filters – 0.45 μ m teflon filter

8.3.2 Pipettes – Class A, glass, appropriate sizes

8.3.3 Pasteur pipettes

8.3.4 Vials – appropriate sizes with Teflon-lined cap

8.3.5 Disposable syringes – plastipak, 5 cc or equivalent

8.3.6 Volumetric flasks – appropriate sizes with ground glass stoppers, Class A.

8.3.7 Vacuum desiccator – glass

8.3.8 Mortar and pestle – ceramic

8.3.9 Sieve – 30 mesh

8.4 Preparation of Material:

8.4.1 Glassware will be washed in a phosphate-free detergent with tap water, followed by tap water rinse, and then a final deionized water rinse. Glassware used with organic solvents will be rinsed with a suitable organic solvent, followed by acetone, followed by tap water and then receive the above listed minimum wash. Prior to use all glassware will be rinsed three times with the solvent in use.

9.0 REAGENTS

9.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

9.1.1 Methanol (CH₃OH), HPLC grade

9.1.2 Tetrabutylammonium Hydroxide (TBA) – HPLC grade, 1.0M in methanol

9.1.3 Organic-free reagent water. All references to water in this method refer to organic-free reagent water, as defined in SW846, Chapter One.

9.2 Stock Standard Solutions

9.2.1 Due to the rarity of some organic colorants, the standards may not be available from commercial sources, and therefore bulk lot samples can be used as standards,

9.2.2 Dry each solid analyte standard to constant weight in a vacuum desiccator in the dark. Place 0.0100 g (weighed to 0.0001 g) of a single analyte into an amber 100 mL volumetric flask and dilute to volume with methanol (for Solvent Red 24 weigh 0.0100 g in 250 mL). Invert flask several times and sonicate for twenty minutes until dissolved. Store in refrigerator at 4 °C in the dark. Calculate the concentration of the stock solutions from the actual weight used. Stock solutions may be used for up to one year.

9.3 Working Standards:

9.3.1 Calibration standards at a minimum of five concentration levels should be prepared for the initial calibration through dilution of the stock standard solutions. Calibration standards should contain multiple target analytes. It is recommended the analytes be grouped to aid in integration. Recommended groupings are as follows: Group 1 – AB45, AY23, AY73, AO10, BY2, DV1, SY33, and DB14; Group 2 – AR64, AB9, BV10, 2AQ, SO3, 1AQ, SY14, and SO7; Group 3 – SY3, BZ, AN, and SR1; Group 4 – DR9, SY2, SG3, and SR24. An example of the dyes concentration ranges can be found in Table 5. These solutions must be refrigerated and stored in the dark. Prepare the working calibration standards as follows:

Standard	Source Solution	Amount Added (ml)	Final Volume (mL)
Standard 5	A	10*	100
Standard 4	B	50	100
Standard 3	C	50	100
Standard 2	D	5	10
Standard 1	D	25	100

*add 20 mL for solvent red 24 and solvent green 3

Source Solution
A = Stock Standard (Each Dye)
B = Standard 5
C = Standard 4
D = Standard 3

9.3.2 For continuing calibration, run a midpoint standard of all dyes at the beginning of the day, and run one group of midpoint standards every 10 samples.

9.4 Surrogate Spiking Solutions

9.4.1 The analyst should monitor the performance of the extraction and analytical system as well as the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard and reagent water blank with two surrogates (i.e., analyte not expected to be present in the sample). The surrogates selected for this method are benzanthrone and anthracene. Both are very strong UV absorbing analyte, yet are somewhat colorless.

9.5 Matrix Spiking Solutions

9.5.1 Prepare matrix-spiking solutions in methanol such that the concentration in the sample is ten times the MDL (See Tables 3 & 4). The matrix spiking solution will be made from the stock standard of the individual analytes chosen.

9.6 HPLC Mobile Phase

9.6.1 To prepare 1 L of 0.005M TBA mobile phase, dilute 5ml of 1.0M TBA in methanol to 1 L with methanol in a volumetric flask

10.0 SAMPLE COLLECTION, PRESERVATION, HANDLING AND RECEIPT

10.1 Upon sample receipt, the laboratory's sample custodian examines each cooler's custody seals to verify that they are intact and that the integrity of the environmental samples has been maintained. The sample custodian signs the COC report. The sample custodian then opens the cooler and measures its internal temperature. The temperature reading is noted on the accompanying COC report. The sample custodian then examines the contents of the cooler. Sample container breakages or discrepancies between the COC report and sample label documentation are recorded. All problems or discrepancies noted during this process are to be promptly related to the field team leader. Inter-laboratory COC procedures and specific procedures for sample handling, storage, disbursement for analysis, and remnant disposal will be followed as specified in the CR4052-PD-0500.

10.2 Samples are collected in amber glass containers with Teflon-lined caps. All samples and sample extracts are stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$. All samples and sample extracts must be stored in the dark until analysis to avoid decomposition of the dyes. Based on guidelines

listed in SW-846 for the extraction of non-volatile compounds, aqueous samples must be extracted within 7 days of collection and solid samples must be extracted within 14 days of collection. All extracts must be analyzed within 40 days of sample preparation.

11.0 PROCEDURE

11.1 Sample Preparation

11.1.1 Aqueous Samples: Solid-phase extraction

- 11.1.1.1 Using a 1 L volumetric flask, add 5 mL of tetrabutylammonium hydroxide (in methanol) by pipette.
- 11.1.1.2 Dilute to volume with aqueous sample. Mix the contents well before extraction.
- 11.1.1.3 If the sample contains sediment, pour the contents of the 1 L volumetric flask into a 1 L beaker and allow the sediment to settle to the bottom. Pour the water portion onto the solid phase extraction disk first, then add the sediment after the water has been pulled through but prior to extraction with methanol.
- 11.1.1.4 Pull sample through the C18 solid phase extraction disk using the vacuum manifold apparatus. A flow rate of 10 to 25 mL/min through the SPE disk is optimal for target analyte recovery. Record the flow rate through the extraction disk in the laboratory notebook.
- 11.1.1.5 Dry the disk using the vacuum manifold apparatus.
- 11.1.1.6 Switch the stopcock to the receiving vessel and pull through a minimum of 30 mL of methanol.
- 11.1.1.7 Evaporate to 1 mL using a rotary evaporator.
- 11.1.1.8 Transfer extract to a 10 mL volumetric flask. Rinse the round bottom flask with methanol and transfer rinse to the 10 mL volumetric flask. Dilute with methanol to volume.

11.1.2 Soil and Sediment Samples

- 11.1.2.1 Dry the soil samples in air at room temperature (or colder) to a constant weight, being careful not to expose the samples to direct sunlight. Grind and homogenize the dried sample thoroughly in an acetonitrile-rinsed mortar to pass a No. 18 sieve.
- 11.1.2.2 Place a 10.0-g sub-sample of each soil sample in a 100-mL volumetric flask. Add 1.0 mL of

surrogate spike solution. If the sample is a matrix spike/matrix spike duplicate, add 1.0 mL of the matrix spike solution. Add 5.0 mL of tetrabutylammonium hydroxide (in methanol) and 5.0 mL organic-free water. Place the stopper on the flask, shake for 1 minute, then place in a cooled ultrasonic bath for 0.5 hour.

11.1.2.3 After sonication, shake sample for 1 minute, then add 50.0 mL of methanol. Shake for 1 minute then place in a cooled ultrasonic bath for 1.0 hour.

11.1.2.4 Transfer approximately 4 mL of the slurry in a disposable syringe and filter it through a 0.45-micron nylon filter. Discard the first 1 mL and retain the remainder in a Teflon-capped vial for HPLC analysis.

11.2 Chromatographic Conditions: Short Gradient

11.2.1 Recommended Column: Waters Xterra MS, C18 column, 4.6mm x 100mm, 3.5um particle size

11.2.2 Mobile Phase: High Purity Water / 0.005 M Tetrabutylammonium Hydroxide (TBA) in Methanol

11.2.3 Dual Gradient: Run time = 35 min

11.2.3.1 Initial Parameters:

75:25 Water:TBA

1.0 mL/min

Column Temp = 30°C

11.2.3.2 Final Parameters:

0:100 Water:TBA

1.0 mL/min

Column Temp = 30°C

11.2.4 Mobile phase flow and composition are varied on a linear gradient throughout the analytical run. The instrument is allowed to come to equilibrium after returning to the initial parameters for 5 min prior to the next run.

11.2.5 Detector Conditions: PDA detector is set to scan from 211nm to 700nm. To maximize sensitivity, extract and integrate a separate chromatogram for each dye at the appropriate, most sensitive, analytical wavelength according to the following chart:

Water Soluble Dyes

Dye Name	Abbr.	Analytical Wavelength (nm)

Acid Blue 1	AB1	635
Acid Blue 9	AB9	628
Acid Blue 45	AB45	633
Acid Orange 10	AO10	485
Acid Red 64	AR64	524
Acid Yellow 3	AY3	383
Acid Yellow 23	AY23	404
Acid Yellow 73	AY73	493
Basic Violet 10	BV10	549
Basic Yellow 2	BY2	378

Organic Soluble Dyes

Dye Name	Abbr.	Analytical Wavelength (nm)
Anthracene	AN	251
Benzanthrone	BZ	399
Disperse Blue 14	DB14	638
Disperse Red 9	DR9	513
Disperse Violet 1	DV1	588
Solvent Green 3	SG3	635
Solvent Orange 3	SO3	408
Solvent Orange 7	SO7	499
Solvent Red 1	SR1	504
Solvent Red 24	SR24	519
Solvent Yellow 2	SY2	416
Solvent Yellow 3	SY3	385
Solvent Yellow 14	SY14	486
Solvent Yellow 33	SY33	381
1-Aminoanthraquinone	1AQ	481
2-Aminoanthraquinone	2AQ	299

Example UV/VIS spectra of each dye and its approximate retention time can be found in Attachment 1

11.3 Chromatographic Conditions: Long Gradient

11.3.1 Recommended Column: Waters Xterra MS, C18 column, 4.6mm x 100mm, 3.5um particle size

11.3.2 Mobile Phase: High Purity Water/0.005 M Tetrabutylammonium Hydroxide (TBA) in Methanol

11.3.3 Dual Gradient: Run time = 65 min

11.3.3.1 Initial Parameters:
75:25 Water:TBA
0.7 mL/min
Column Temp = 30°C

11.3.3.2 Final Parameters:
0:100 Water:TBA
1.0 mL/min

Column Temp = 30°C

11.3.4 Mobile phase flow and composition are varied on a linear gradient throughout the analytical run. The instrument is allowed to come to equilibrium after returning to the initial parameters for 5 min prior to the next run.

11.3.5 Detector Conditions: Same as those described in Section 11.2.5.

11.4 Calibration of HPLC:

The entire HPLC system is allowed to warm up for a minimum of 30 minutes. During this time the mobile phase, at the initial gradient conditions, should be pumped through the column.

11.4.1 Initial Calibration

11.4.1.1 Injections of each calibration standard over the concentration range of interest are performed. Peak heights and peak areas are obtained for each analyte at the appropriate wavelength.

11.4.2 Daily Calibration

11.4.2.1 Analyze the midpoint calibration standards along with an instrument blank at the beginning of each day and after each set of 10 samples or less. Obtain the response factor for each analyte from the mean peak heights or peak areas and compare it with the response factor obtained for the initial calibration. The mean response factor for the daily calibration must agree within $\pm 25\%$ of the response factor of the initial calibration. The same criterion is required for subsequent standard responses. If this criterion is not met, a new initial calibration must be obtained. Note exemption in Section 2.8.

11.5 HPLC Analysis

11.5.1 Analytical Sequence

All acceptable samples must be analyzed within a valid analysis sequence as given below:

Initial Calibration:

Injection Number	Material Injected
1	Instrument Blank

2 – 21	Five Initial Calibration Standards
22	Instrument Blank at end of initial calibration

Daily Analysis Sequence:

Time	Injection Number	Material Injected
0hr	1	Instrument blank
	2 – 5	Midpoint standard of all dyes (ICV)
	6	Instrument Blank
	7	LCS
	8 – 18	Sample Analyses
~12 hr	19	Instrument Blank
	20	Mid-level CCV of one group of dyes
	21	LCS
	22 – 32	Sample Analyses
~12 hr	33	Instrument Blank
	34	Mid-level CCV of one group of dyes
	Etc.	

After the initial calibration, the analysis sequence may continue as long as acceptable instrument blanks and continuing calibration verifications are analyzed at the required frequency. This analysis sequence shows only the minimum required blanks and standards. More blanks and standards may be run at the discretion of the analysts; these must also satisfy the criteria presented in Section 12 in order to continue the run sequence.

An analysis sequence must include all required matrix spike/matrix spike duplicate and method blank analyses. The analysts may decide at what point in the sequence they are to be analyzed.

11.5.2 Laboratory Control Sample (LCS)

11.5.2.1 The LCS (reagent blank spike) should be prepared

separately from the standards and include at least 6 of the dyes, 3 water soluble and 3 organic soluble. The concentration of the dyes should be approximately 500 µg/L.

11.5.3 Follow the guidelines found in SW846, Method 8000 for the following parameters.

11.5.3.1 Retention Windows

11.5.3.1.1 Retention time windows are established by performing 7 replicate injections over a 72

hour period. Retention time windows are set at 3 times the standard deviation of the replicate injections.

11.5.3.2 Any modifications to the RTW

11.5.3.2.1 If the retention time of any analyte in the standards at the beginning of the analytical shift does not fall within the ± 3 standard deviation window, then a new initial calibration is necessary unless system maintenance corrects the problem.

11.5.3.3 Initial Calibration

11.5.3.3.1 The initial calibration involves the analysis of standards containing each one of the analytes at five concentrations over the working range of the instrument.

11.5.3.4 Initial Calibration Verification

11.5.3.4.1 The initial calibration is verified at the beginning of each 12-hour analytical shift. The process involves the analysis of the mid-point standard for each group of dyes. If the response for the analyte is within $\pm 25\%$ of the response obtained during the initial calibration, then the initial calibration is considered still valid. Note exemption in Section 2.8.

11.5.3.5 Instrument Blank

11.5.3.5.1 An instrument blank is analyzed prior to injection of the standards, after the analysis of all standards, and after every 10 samples.

11.5.3.6 Continuing Calibration Verification

11.5.3.6.1 The continuing calibration verification process involves the analysis of the mid-point standard for one group of dyes. If the response for the analyte is within $\pm 25\%$ of the response obtained during the initial calibration, then the initial calibration is considered still valid. The CCV is analyzed every 10 samples. Note exemption in Section 2.8.

11.5.3.7 Sample Analysis

11.5.3.7.1 An autosampler is used to inject 10 to 100 μL of a sample. Samples are analyzed according to the sequence in 11.5.1.

11.5.4 Compound Identification

11.5.4.1 Compound identification is achieved through spectral matching with known standards of the dyes.

11.5.4.2 In the event of co-elution problems, the analyst will attempt to separate the co-eluting compounds using either the short or the long gradient as appropriate. Re-analysis of the sample to address co-elution problems will occur only if a target dye is positively identified (by retention time and spectra) and is co-eluting with another positively identified target dye. All samples must be analyzed within a valid analysis sequence as defined in Section 11.5.

11.5.5 Compound Quantification

11.5.5.1 Data Acquisition, Calculations and Data Reduction

11.5.5.1.1 All data acquisition, chromatogram integration, calibration curve calculation and unknown concentration calculation is preformed utilizing the Waters Millennium³² chromatography software.

11.5.5.1.2 Manual calculations such as standard deviation and percent recovery will be performed utilizing a spreadsheet.

11.5.6 Data Management and Records Management

11.5.6.1 All documents are managed according to CR4052-PD-0100 and CR4052-PD-0150. Raw data is stored on using the Millennium 32 chromatography software. The data is backed-up monthly.

12 QUALITY CONTROL AND QUALITY ASSURANCE

12.1 Initial Calibration

12.1.1 Criteria: Analyze standards at five concentrations over working range of instrument. Linearity is determined by an $RSD \leq 30\%$ or a coefficient of determination greater or equal to 0.99

12.1.2 Corrective Action: If the initial calibration does not meet the criteria for linearity, reduce/increase the concentration of the upper/lower standards until the criteria is meet.

12.2 Initial Calibration Verification

- 12.2.1 Criteria: The initial calibration is verified at the beginning of each 12-hour analytical shift. A mid-point standard for each group of dyes is analyzed. If the response for the analyte is within $\pm 25\%$ of the response obtained during the initial calibration, then the initial calibration is considered still valid. Up to 5 dyes are allowed to be outside this tolerance for the initial calibration to be considered still valid. Note exemption in Section 2.8.
- 12.2.2 Corrective Action: If the initial calibration is no longer valid, a new initial calibration must be performed unless system maintenance corrects the problem.
- 12.3 Continuing Calibration Verification
- 12.3.1 Criteria: The continuing calibration verification process involves the analysis of a mid-point standard for one group of dyes. If the response for the analyte is within $\pm 25\%$ of the response obtained during the initial calibration, then the initial calibration is considered still valid. Up to 2 dyes are allowed to be outside this tolerance for the initial calibration to be considered still valid. The CCV is analyzed every 10 samples. Note exemption in Section 2.8.
- 12.3.2 Corrective Action: If the initial calibration is no longer valid, a new initial calibration must be performed unless system maintenance corrects the problem.
- 12.4 Instrument Blank
- 12.4.1 Criteria: An instrument blank is analyzed prior to injection of the standards, after the analysis of all standards, and after every 10 samples. The results of the instrument blank should be less than the IDL.
- 12.4.2 Corrective Action: If the instrument blank does not meet the acceptance criteria, then the source of the contamination will be located and reduced. The samples associated with the contaminated instrument blank will be re-analyzed.
- 12.5 Method Blank
- 12.5.1 Criteria: A clean sample will be extracted using the appropriate procedure and analyzed. The results of the method blank should be less than the MDL or less than 5% of the sample result for the same analyte. A method blank will be prepared for each group of 20 samples.
- 12.5.2 Corrective Action: If the method blank does not meet the acceptance criteria, then the source of the contamination will be located and reduced. The samples associated with the contaminated method blank will be re-extracted and re-analyzed.

12.6 Laboratory Control Sample

12.6.1 Criteria: A clean matrix similar to the sample matrix and of the same weight or volume will be spiked with the same analytes at the same concentrations as the matrix spike, extracted using the same method, and analyzed. When the results of the matrix spike analysis indicates a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

12.6.2 Corrective Action: If the LCS does not meet the acceptance criteria, the source of the problem will be located and corrected.

12.7 Matrix Spike

12.7.1 Criteria: A sample matrix will be spiked with a minimum of 2 water-soluble dyes, 2 organic-soluble dyes, and the surrogate standards, extracted using the same method, and analyzed. Percent recovery is calculated.

12.7.2 Corrective Action: If the matrix spike indicates there may be interference from the matrix, then the LCS will be used to verify that the laboratory can perform the analysis in a clean matrix.

12.8 Matrix Spike Duplicate

12.8.1 Criteria: The same matrix used for the matrix spike will be spiked with the same analytes at the same concentrations as the matrix spike. Percent recovery is calculated.

12.8.2 Corrective Action: If the matrix spike indicates there may be interference from the matrix, then the LCS will be used to verify that the laboratory can perform the analysis in a clean matrix.

12.9 Surrogate Standards

12.9.1 Criteria: A solution of benzantrone and anthracene will be spiked on the sample matrix. Percent recovery is calculated.

13 REPORTS

13.1 Electronic Deliverables: Final results will be provided in a Microsoft Excel format. The following information will be included in the Excel sheet: sample number, laboratory identification, laboratory name, associated blank, quality control type, sample date, receipt date, extraction date, analysis date, run number, parameter, CAS number, method, laboratory results, instrument detection limits,

method detection limits, percent moisture, percent recovery of surrogates, and comments. Another Excel sheet will report RSD and coefficients of determination for the initial calibration curves, and tabulated results of the daily and continuing calibration standards.

- 13.2 Full data package: In addition to the electronic deliverables, the full data package will include chromatograms, HPLC concentration results, and UV-Vis spectra for the initial calibration, daily calibration, continuing calibration, instrument blanks, method blanks, and each sample. Full package will be provided when there is a positive detection of a target analyte.
- 13.3 Abbreviated data package: In addition to the electronic deliverables, the field screening data package will include chromatograms and HPLC concentration results for the initial calibration, daily calibration, and continuing calibration. Chromatograms for the samples will be provided to prove the absence of detectable dyes. The abbreviated data package will be provided when no target analyte is detected in the sample.
- 13.4 If an analyte is noted on the chromatogram but can not be confirmed by its UV-Vis spectra, then "NSC" (no spectra confirmation) will be noted in the comments section of the Electronic Deliverables

14 REFERENCES

- 14.2 Wegener, J.W., J.C. Klamer, H. Govers, and U.A. Th. Brinkman (1987) Determination of Organic Colorants in Cosmetic Products by High Performance Liquid Chromatography.
- 14.3 Chromatographia, 24: 865-875. Garrison, A.W., G.L. Baughman, E.J. Weber, R.L. Adams, and M.S. Brewer (1992) Fate of Colored Smoke Dyes. Army Project Order 88PP8863.
- 14.4 Alcantara-Licudine, J.P., M.K. Kawate, and Q.X. Li (1997) Method for the Analysis of Phloxine B, Uranine, and Related Xanthene Dyes in Soil Using Supercritical Fluid Extraction and High Performance Liquid Chromatography. Journal of Agricultural Food Chemistry, 45: 766-773.
- 14.5 Gagliardi, L., G. Cavazzutti, A. Amato, A. Basili, and D. Tonelli (1987) Identification of Cosmetic Dyes by Ion Pair Reversed Phase High Performance Liquid Chromatography. Journal of Chromatography, 394: 345-352.
- 14.6 Eakes, W., J. Heath, M. Cavit, K. Rimm, W. Powers, C. Connelly, J. Anderson, M. Johnson (1983) Initial Assessment Study of Naval Weapons Support Center Crane, Indiana. NEESA 13-003.
- 14.7 U.S. Environmental Protection Agency, Method SW-846-8330.
- 14.8 U.S. Environmental Protection Agency, Method SW-846-8000.
- 14.9 U.S. Environmental Protection Agency, Method SW-846-3500.
- 14.10 U.S. Environmental Protection Agency, Method SW-846-3550.

15 ATTACHMENTS

CR4052-PD-2500(1) Stock Standards Data Sheet

CR4052-PD-2500(2) Working Standards Data Sheet

CR4052-PD-2500(3) Surrogate and Matrix Spike Data Sheet

CR4052-PD-2500(4) Aqueous Extraction Data Sheet

CR4052-PD-2500(5) Solid Extraction Data Sheet

16 REVISION BLOCK

REV	DATE	REVISION	AUTHOR
0	5/09/01	Original Issue	JJK

TABLE 1
Quality Control Limits
Matrix Spike/Matrix Spike Duplicate Samples and Surrogate Spikes
Dye Analyses by the Short Gradient
Naval Surface Warfare Center
Crane, Indiana

Chemical	Solid Matrix	
	Accuracy (%R)	Precision (RPD)
Acid Blue 9	3-9	35
Acid Blue 45	46-75	35
Acid Orange 10	191-213	35
Acid Red 64	61-69	35
Acid Yellow 23	55-74	35
Acid Yellow 73	61-68	35
1-Aminoanthraquinone	73-94	35
2-Aminoanthraquinone	42-46	35
Basic Violet 10	94-105	35
Disperse Blue 14	34-73	35
Disperse Red 9	40-48	35
Disperse Violet 1	45-68	35
Solvent Green 3	25-72	35
Solvent Orange 3	51-54	35
Solvent Orange 7	58-94	35
Solvent Red 1	25-88	35
Solvent Red 24	TBD	TBD
Solvent Yellow 2	41-47	35
Solvent Yellow 3	210-225	35
Solvent Yellow 14	41-44	35
Solvent Yellow 33	35-41	35
Anthracene	42-57	NA
Benzanthrone	99-113	NA

NA : Not applicable

QC limits are based on the seven low spike samples analyzed during method performance/validation. Matrix spike QC limits are the same as laboratory control sample QC limits. Limits are not presented for aqueous samples since they will be quantitated using the long gradient.

TABLE 2
Quality Control Limits
Matrix Spike/Matrix Spike Duplicate Samples and Surrogate Spikes
Dye Analyses by the Long Gradient
Naval Surface Warfare Center
Crane, Indiana

Chemical	Aqueous Matrix ⁽¹⁾	
	Accuracy (%R)	Precision (RPD) ⁽²⁾
Acid Blue 9	16-113	37
Acid Blue 45	0-45	53
Acid Orange 10	50-114	20
Acid Red 64	43-109	22
Acid Yellow 23	48-111	20
Acid Yellow 73	47-110	20
1-Aminoanthraquinone	41-96	20
2-Aminoanthraquinone	36-87	21
Basic Violet 10	46-111	21
Disperse Blue 14	35-109	26
Disperse Red 9	46-115	22
Disperse Violet 1	46-100	20
Solvent Green 3	2-84	47
Solvent Orange 3	41-92	20
Solvent Orange 7	37-65	20
Solvent Red 1	38-74	20
Solvent Red 24	34-102	25
Solvent Yellow 2	47-104	20
Solvent Yellow 3	49-116	20
Solvent Yellow 14	42-88	20
Solvent Yellow 33	46-106	20
Anthracene	0-106	NA
Benzanthrone	32-120	NA

NA: Not Applicable

Solid samples will be quantitated by the short gradient.

1. QC limits based on 11 low spike DI water samples analyzed during Rounds 2 and 3 of the Method Validation Study. New limits for groundwater will be developed when sufficient data has been generated.

2. RPD limits of 20% for aqueous and 35% for solids were substituted for the calculated value in instances where the calculated RPD was less than the 20% or 35% limit.

TABLE 3
Analytical Methods and Limits of Detection
For the Short Gradient
Naval Surface Warfare Center
Crane, Indiana

Chemical	Laboratory MDL		Laboratory RL		Risk-Based Target Level	
	Aqueous (ug/L)	Solid (ug/kg)	Aqueous (ug/L)	Solid (ug/Kg)	Aqueous (ug/L)	Solid (ug/kg)
Acid Blue 1	55.3	558.9	26.63	15978	450	10000
Acid Blue 9	27.5	141.8	22.63	13578	230000	1630000
Acid Blue 45	79.1	791.6	23.25	13950		
Acid Orange 10	39.3	522.7	25.13	15078	100	6460
Acid Red 64	17.1	240.7	62.5	37500		
Acid Yellow 3	104.3	149.0	22.88	13728		
Acid Yellow 23	92.7	403.7	25.13	15078		
Acid Yellow 73	19.7	179.9	22.63	13578		
1-Amino-anthraquinone	18.7	425.6	16.06	9636		
2-Amino-anthraquinone	10.9	138.7	23.36	14016	2	14700
Basic Violet 10	20.8	177.2	19.88	11928	570	12000
Basic Yellow 2	384.6	1350.6	23.13	13878	60	1300
Disperse Blue 14	60.7	912.5	18.69	11214		
Disperse Red 9	20.7	213.1	16.51	9906		
Disperse Violet 1	35.5	467.8	16.26	9756		
Solvent Green 3	45.2	1156.9	18.38	11028		
Solvent Orange 3	28.7	65.3	15.99	9594	600	129000
Solvent Orange 7	97.4	799.3	18.00	10800	730	52000
Solvent Red 1	84.6	2304.2	28.99	17394		
Solvent Red 24	27.9	0	7.1	4260		
Solvent Yellow 2	23.7	192.5	19.95	11970	0.01	110
Solvent Yellow 3	54.1	391.2	18.88	11328	0.02	130
Solvent Yellow 14	33.7	80.7	17.70	10620	460	3230
Solvent Yellow 33	20.5	136.5	17.48	10488		
Anthracene	115.9	331.9	25.96	15576		
Benzanthrone	30.4	176.2	15.2	9120		

The risk-based screening levels were developed by USEPA Region 5 specifically for this project

TABLE 4
Analytical Methods and Limits of Detection
For the Long Gradient
Naval Surface Warfare Center
Crane, Indiana

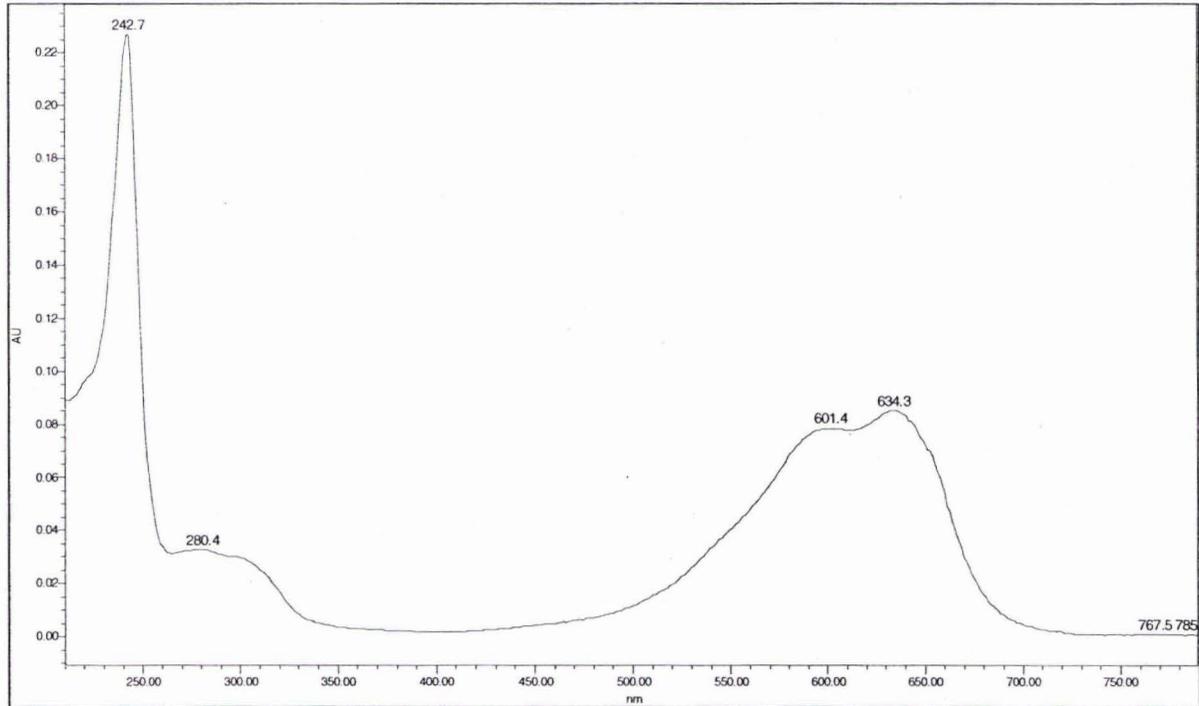
Chemical	Laboratory MDL		Laboratory RL		Risk-Based Target Level	
	Aqueous (ug/L)	Solid (ug/kg)	Aqueous (ug/L)	Solid (ug/Kg)	Aqueous (ug/L)	Solid (ug/kg)
Acid Blue 1	43.8 ⁽¹⁾	523.4	26.63	15978	450	10000
Acid Blue 9	25.1	89.5	22.63	13578	230000	1630000
Acid Blue 45	26.7	407.5	23.25	13950		
Acid Orange 10	13.0	262.0	25.13	15078	100	6460
Acid Red 64	12.1	325.8	62.5	37500		
Acid Yellow 3	24.6 ⁽¹⁾	2029.5	22.88	13728		
Acid Yellow 23	12.8	512.8	25.13	15078		
Acid Yellow 73	13.5	169.0	22.63	13578		
1-Amino-anthraquinone	12.2	242.5	16.06	9636		
2-Amino-anthraquinone	12.6	230.0	23.36	14016	2	14700
Basic Violet 10	15.0	146.0	19.88	11928	570	12000
Basic Yellow 2	170	1334.1	23.13	13878	60	1300
Disperse Blue 14	16.7	390.9	18.69	11214		
Disperse Red 9	14.8	176.7	16.51	9906		
Disperse Violet 1	11.1	950.1	16.26	9756		
Solvent Green 3	18.5	697.9	18.38	11028		
Solvent Orange 3	11.7	56.7	15.99	9594	600	129000
Solvent Orange 7	6.1	176.2	18.00	10800	730	52000
Solvent Red 1	7.9	2295.6	28.99	17394		
Solvent Red 24	2.9		7.1	4260		
Solvent Yellow 2	11.8	221.0	19.95	11970	0.01	110
Solvent Yellow 3	16.2	194.6	18.88	11328	0.02	130
Solvent Yellow 14	13.5	94.7	17.70	10620	460	3230
Solvent Yellow 33	13.7	137.4	17.48	10488		
Anthracene	18.4	236.6	25.96	15576		
Benzanthrone	13.1	83.1	15.2	9120		

The risk-based screening levels were developed by USEPA Region 5 specifically for this project.

1. Values determined during Round 1. All other values determined during Rounds 2 and 3 of the Method Validation Study.

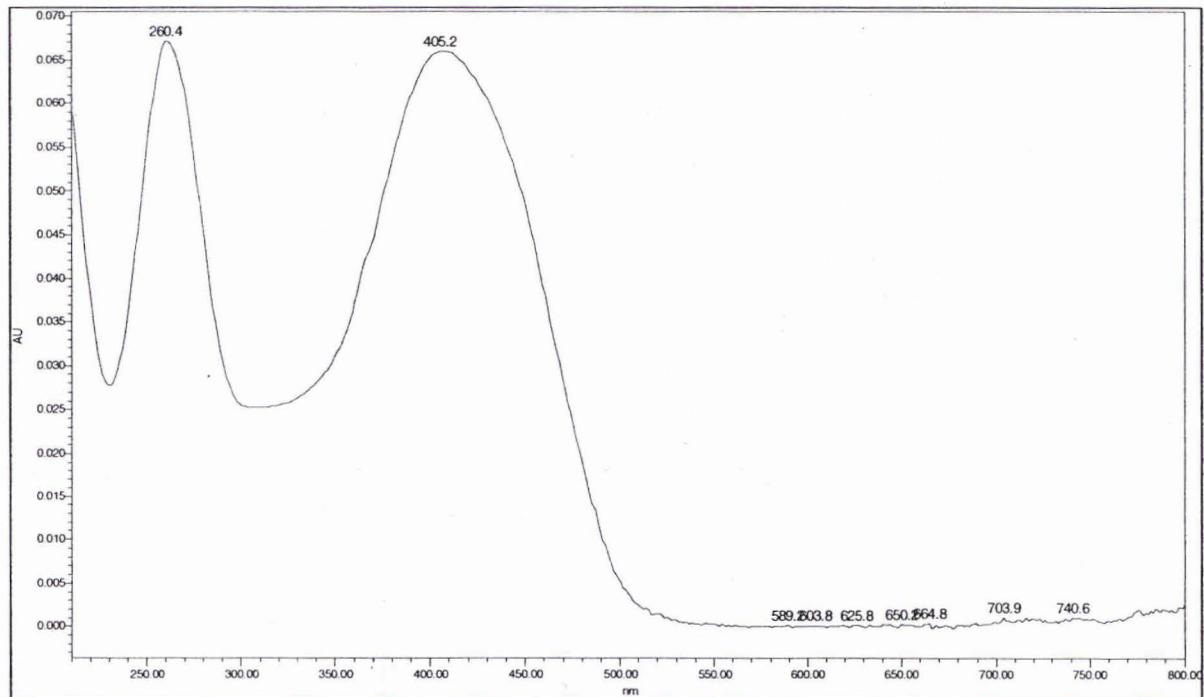
Table 5
Example Standard Concentrations for the Initial Calibration

Dye	Level 5 (ug/mL)	Level 4 (ug/mL)	Level 3 (ug/mL)	Level 2 (ug/mL)	Level 1 (ug/mL)
Acid Blue 45	37.200	18.600	9.300	4.650	2.325
Acid Yellow 23	40.200	20.100	10.050	5.025	2.513
Acid Yellow 73	36.200	18.100	9.050	4.525	2.263
Acid Orange 10	40.200	20.100	10.050	5.025	2.513
Acid Blue 1	42.600	21.300	10.650	5.325	2.663
Basic Yellow 2	37.000	18.500	9.250	4.625	2.313
Disperse Violet 1	26.020	13.010	6.505	3.253	1.626
Solvent Yellow 33	27.960	13.980	6.990	3.495	1.748
Disperse Blue 14	29.900	14.950	7.475	3.738	1.869
Acid Red 64	50.800	40.640	25.400	12.700	6.250
Acid Blue 9	36.200	18.100	9.050	4.525	2.263
Basic Violet 10	31.800	15.900	7.950	3.975	1.988
2- Aminoanthraquinon e	37.380	18.690	9.345	4.673	2.336
Solvent Orange 3	25.580	12.790	6.395	3.198	1.599
1- Aminoanthraquinon e	25.700	12.850	6.425	3.213	1.606
Solvent Yellow 14	28.320	14.160	7.080	3.540	1.770
Solvent Orange 7	28.800	14.400	7.200	3.600	1.800
Acid Yellow 3	36.600	18.300	9.150	4.575	2.288
Solvent Yellow 3	30.200	15.100	7.550	3.775	1.888
Benzanthrone	24.320	12.160	6.080	3.040	1.520
Anthracene	41.540	20.770	10.385	5.193	2.596
Solvent Red 1	46.380	23.190	11.595	5.798	2.899
Disperse Red 9	26.420	13.211	6.605	3.303	1.651
Solvent Yellow 2	31.920	15.960	7.980	3.990	1.995
Solvent Green 3	29.400	14.700	7.350	3.675	1.838
Solvent Red 24	5.680	2.840	1.420	1.136	0.710

ATTACHMENT 1: SPECTRA AND APPROXIMATE RETENTION TIMES

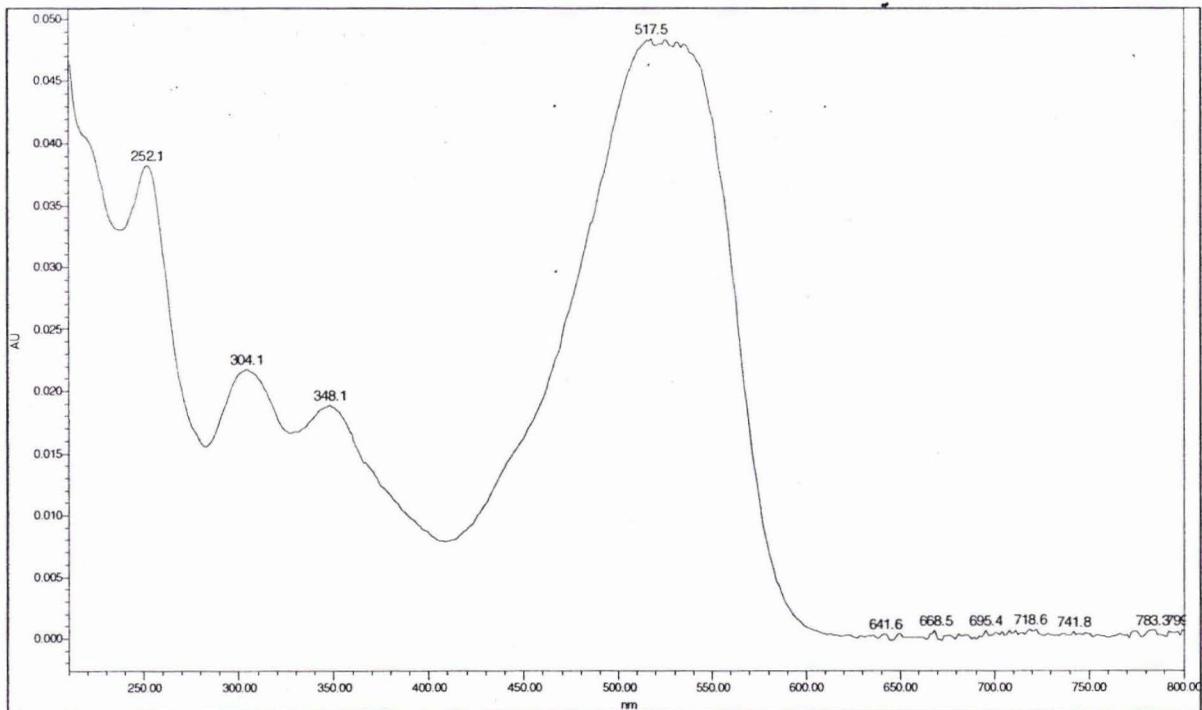
AB45 RT (SHORT GRAD) = 4.4 ± 2.0

AB45 RT (LONG GRAD) = 6.8 ± 10.6



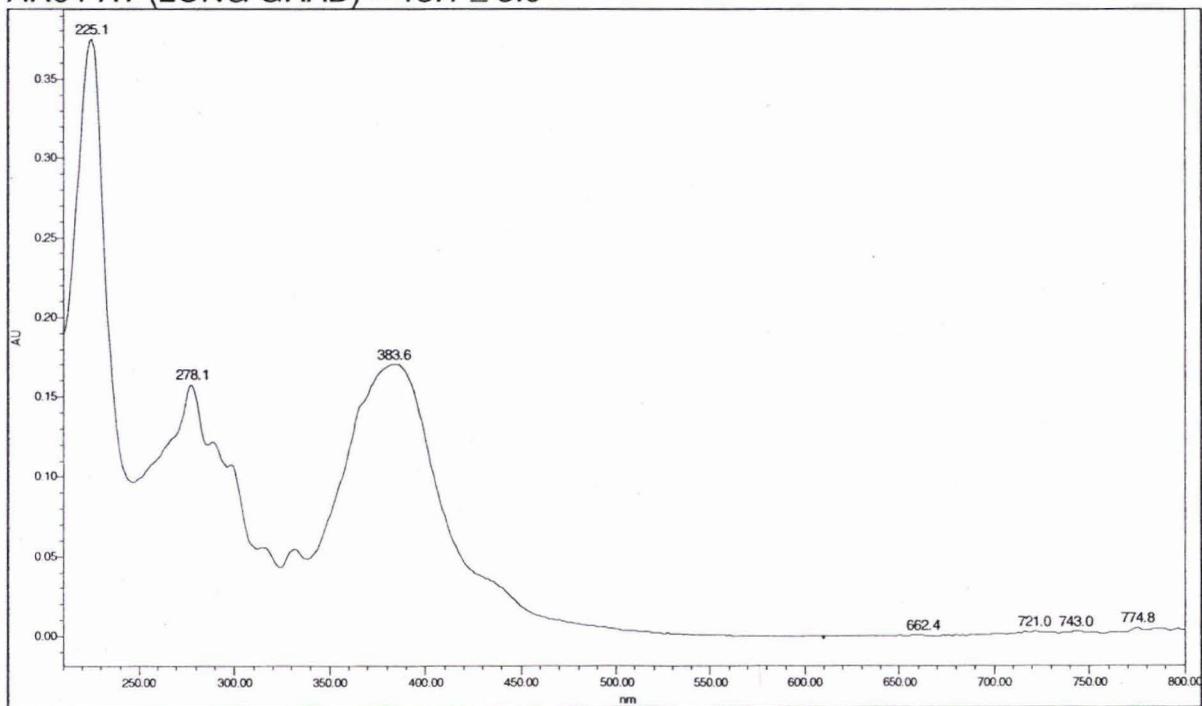
AY23 RT (SHORT GRAD) = 6.8 ± 3.3

AY23 RT (LONG GRAD) = 14.1 ± 5.2



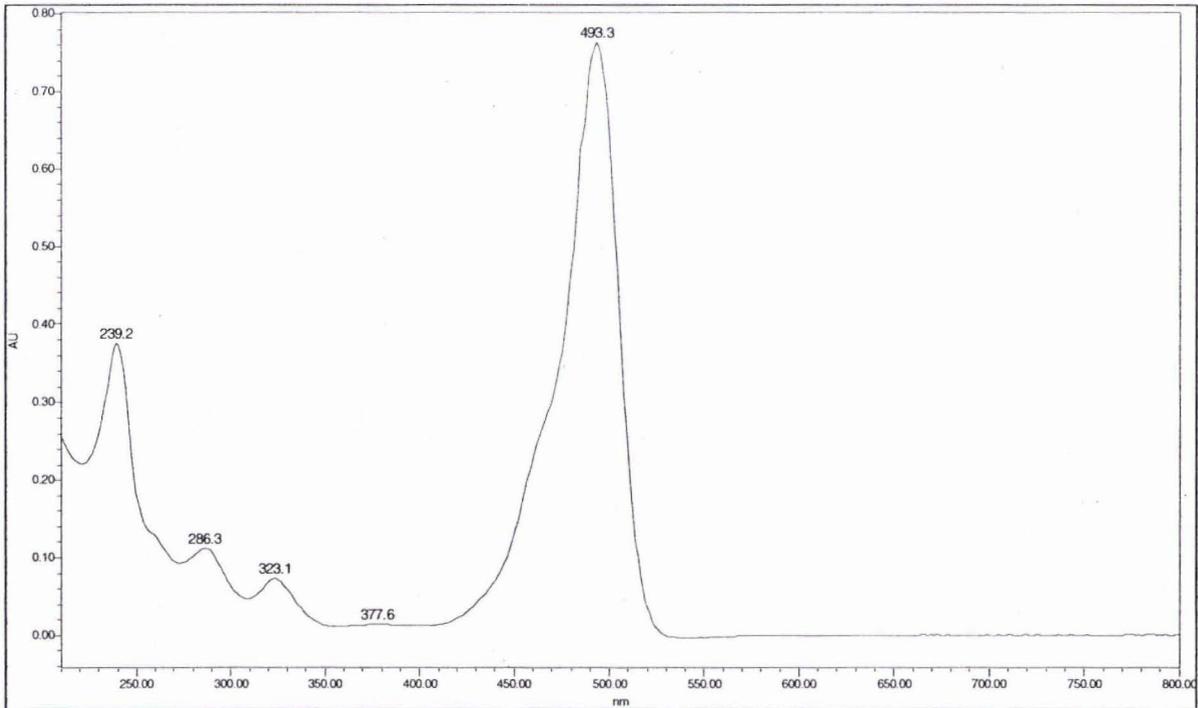
AR64 RT (SHORT GRAD) = 6.2 ± 2.7

AR64 RT (LONG GRAD) = 13.1 ± 3.0

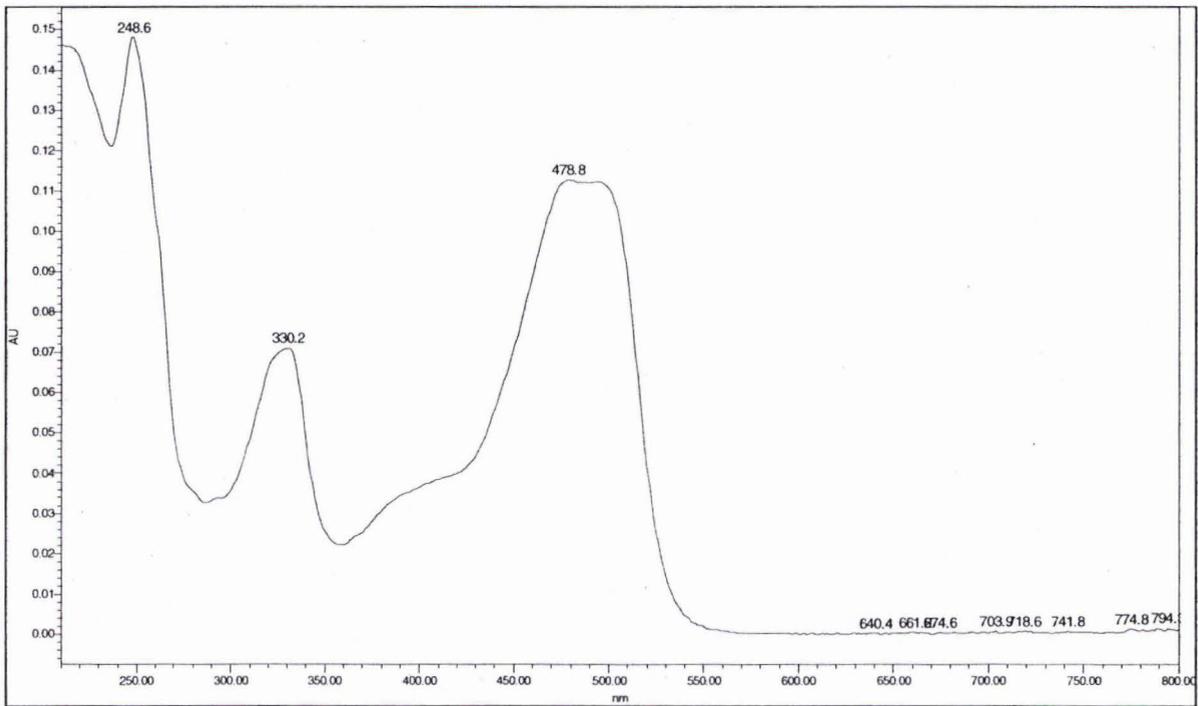


AY3 RT (SHORT GRAD) = 6.8 ± 2.9

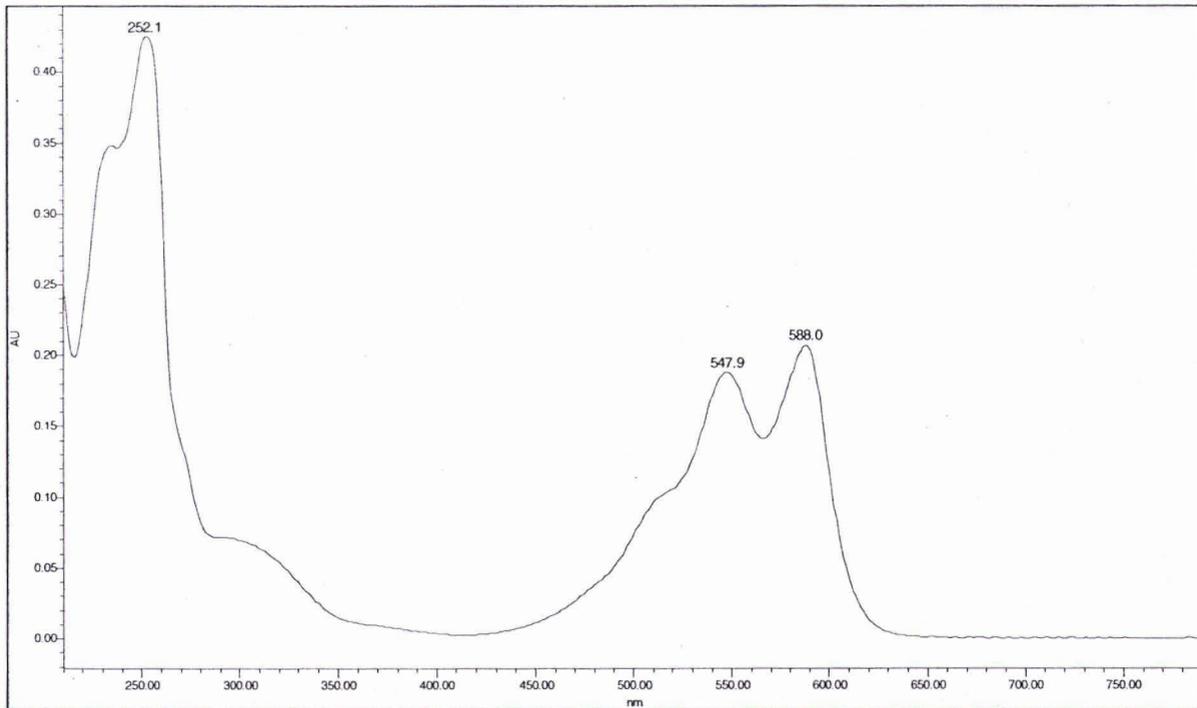
AY3 RT (LONG GRAD) = 13.7 ± 3.4



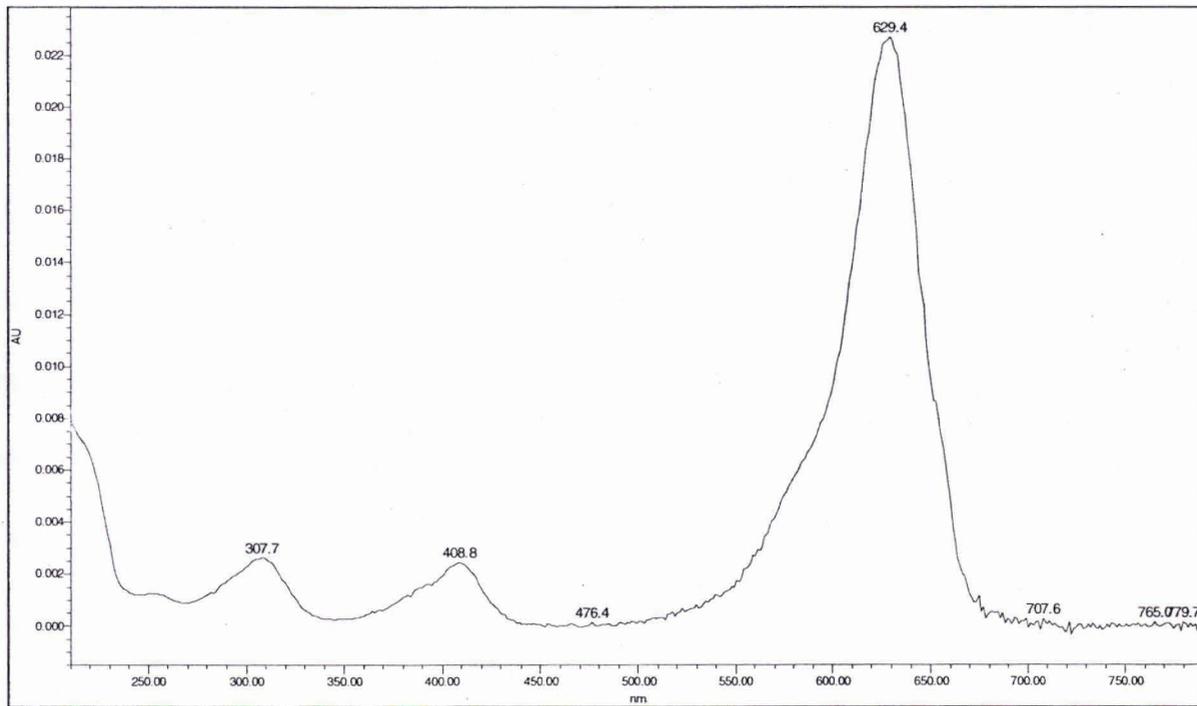
AY73 RT (SHORT GRAD) = 8.6 ± 2.3
AY73 RT (LONG GRAD) = 17.0 ± 4.0



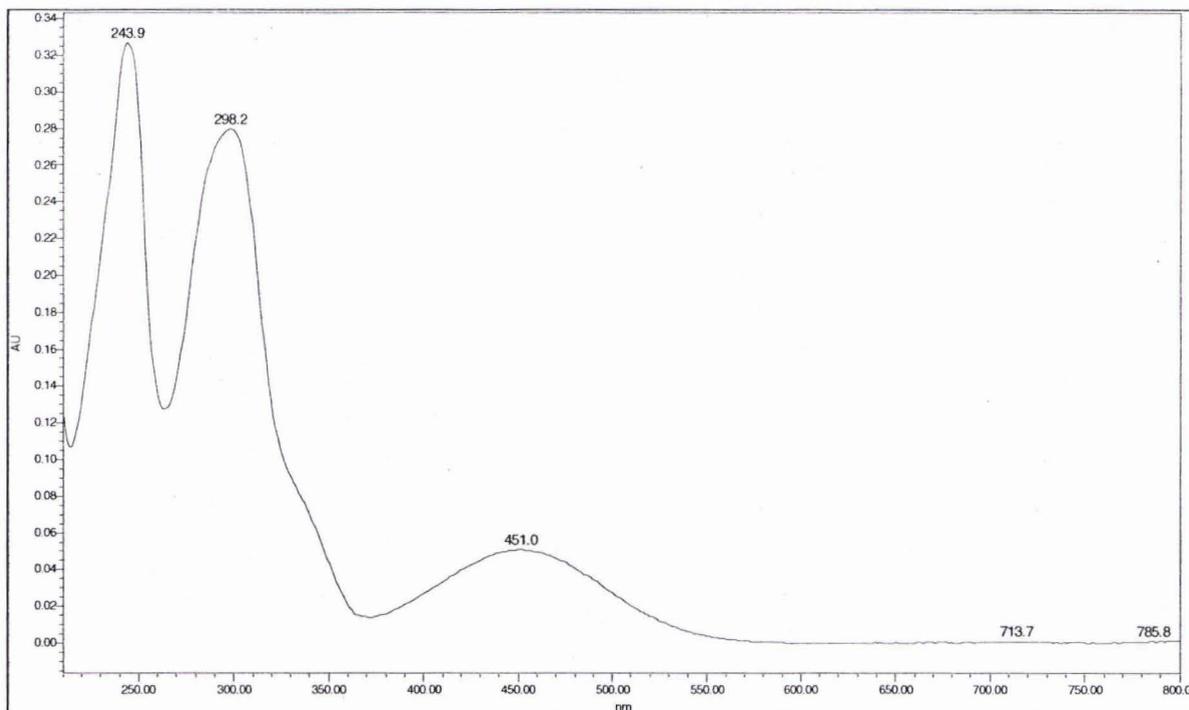
AO10 RT (SHORT GRAD) = 9.5 ± 2.1
AO10 RT (LONG GRAD) = 18.4 ± 3.6



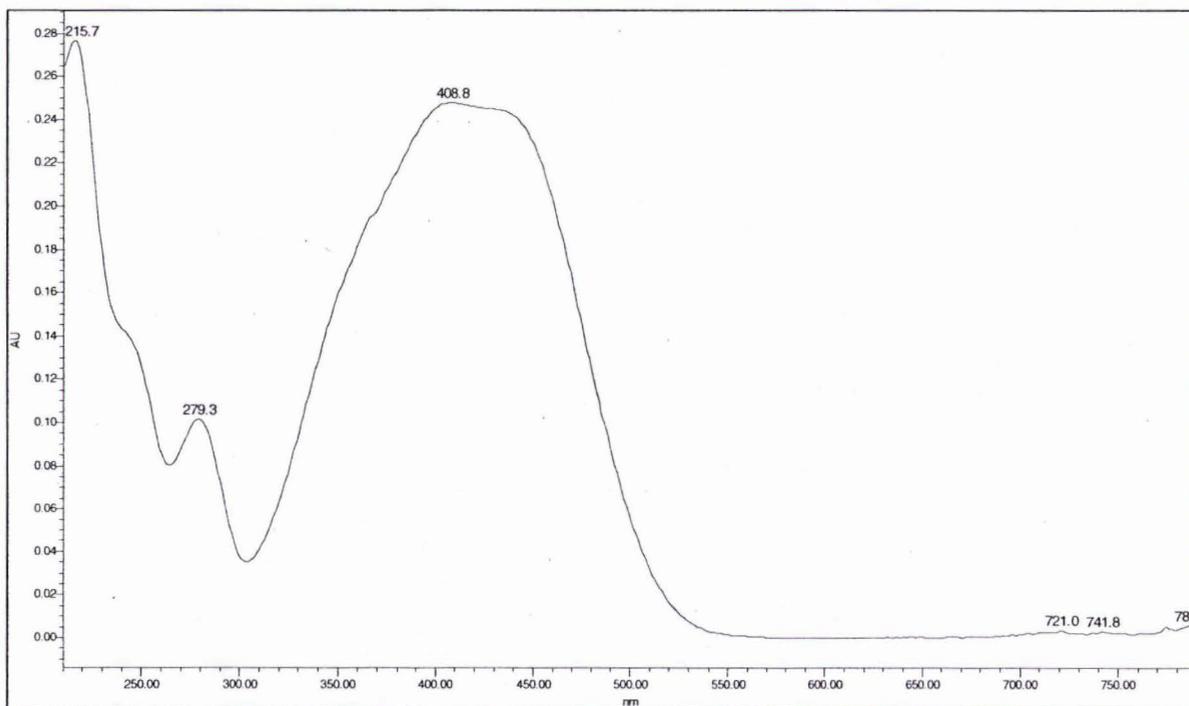
DV1 RT (SHORT GRAD) = 12.7 ± 0.2
DV1 RT (LONG GRAD) = 22.8 ± 0.2



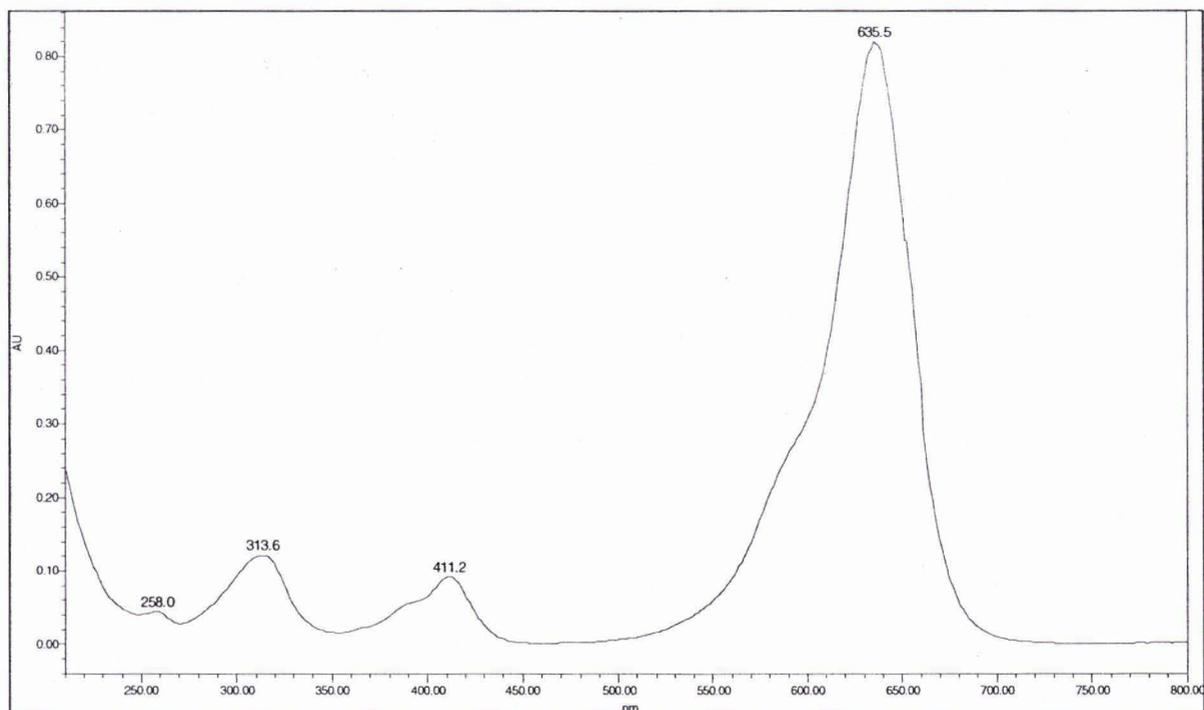
AB9 RT (SHORT GRAD) = 11.3 ± 1.4
AB9 RT (LONG GRAD) = 21.8 ± 2.6



2AQ RT (SHORT GRAD) = 13.2 ± 0.5
2AQ RT (LONG GRAD) = 23.8 ± 0.5

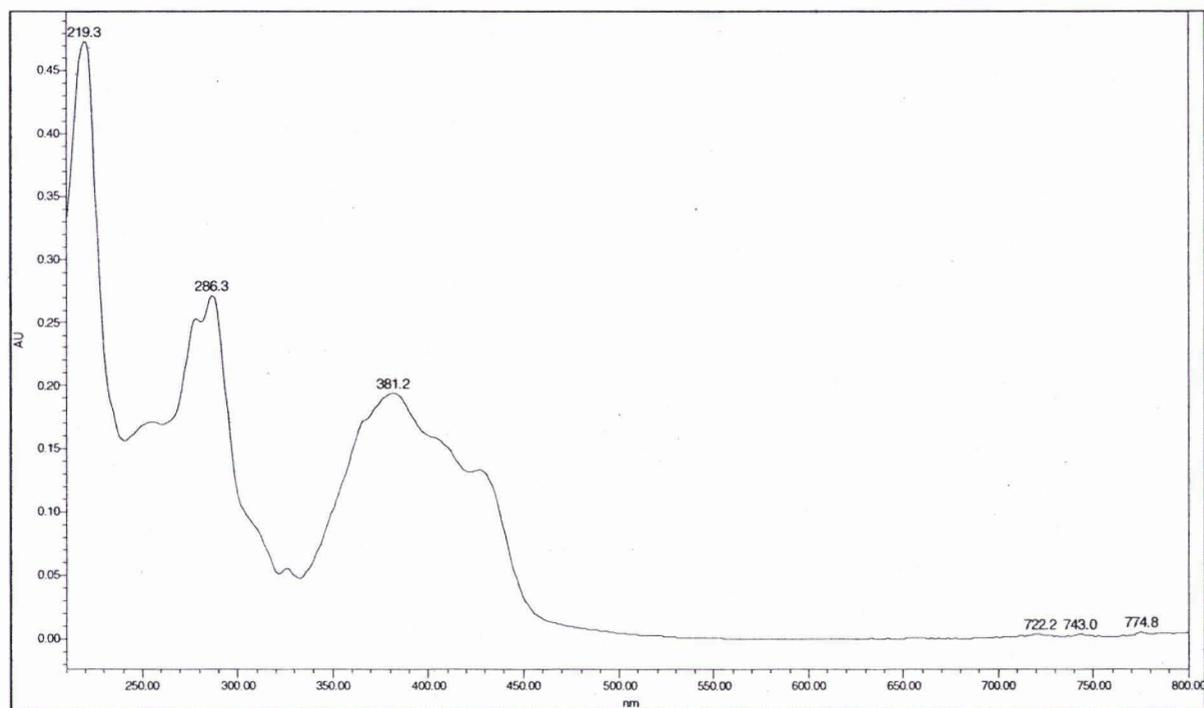


SO3 RT (SHORT GRAD) = 13.8 ± 0.2
SO3 RT (LONG GRAD) = 24.8 ± 0.2



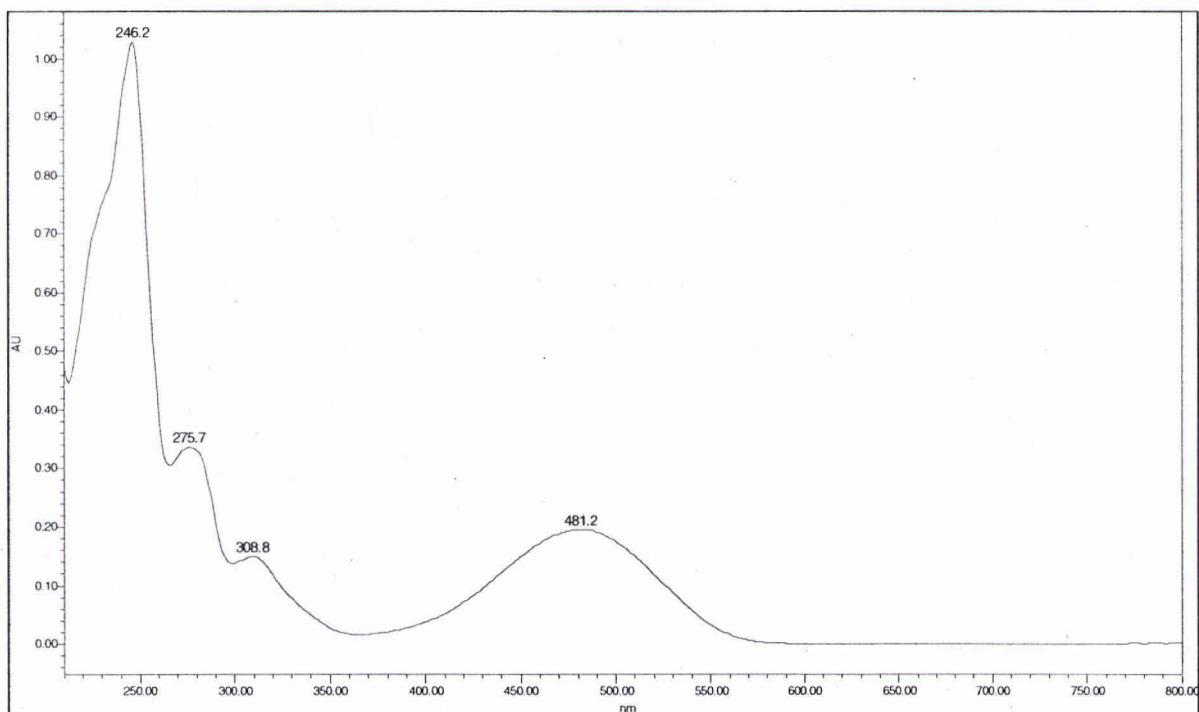
AB1 RT (SHORT GRAD) = 12.4 ± 1.5

AB1 RT (LONG GRAD) = 23.5 ± 2.3



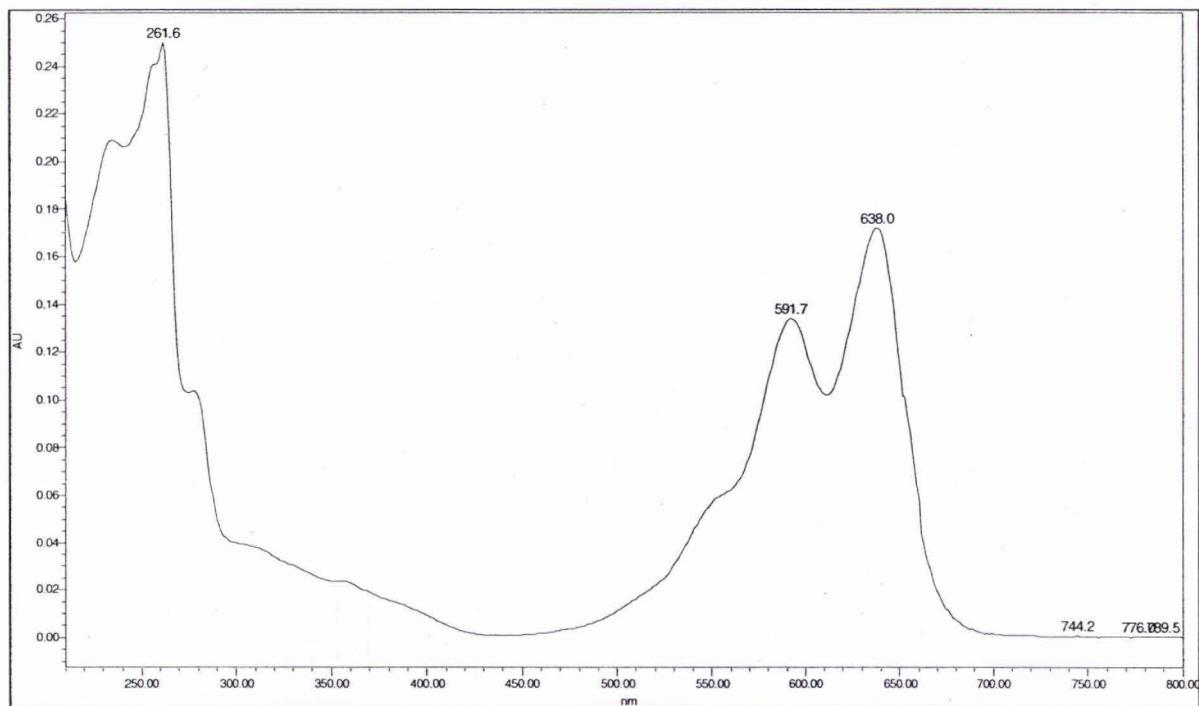
SY33 RT (SHORT GRAD) = 15.3 ± 0.8

SY33 RT (LONG GRAD) = 28.1 ± 1.2



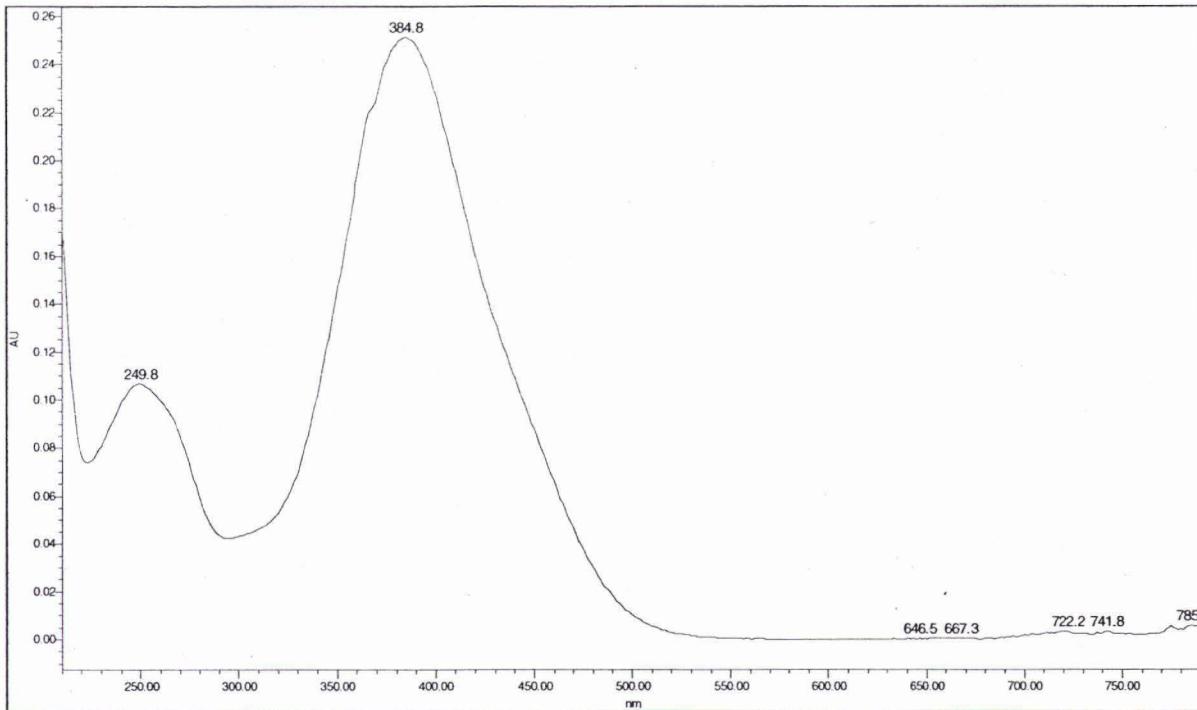
1AQ RT (SHORT GRAD) = 16.0 ± 0.6

1AQ RT (LONG GRAD) = 28.9 ± 0.9



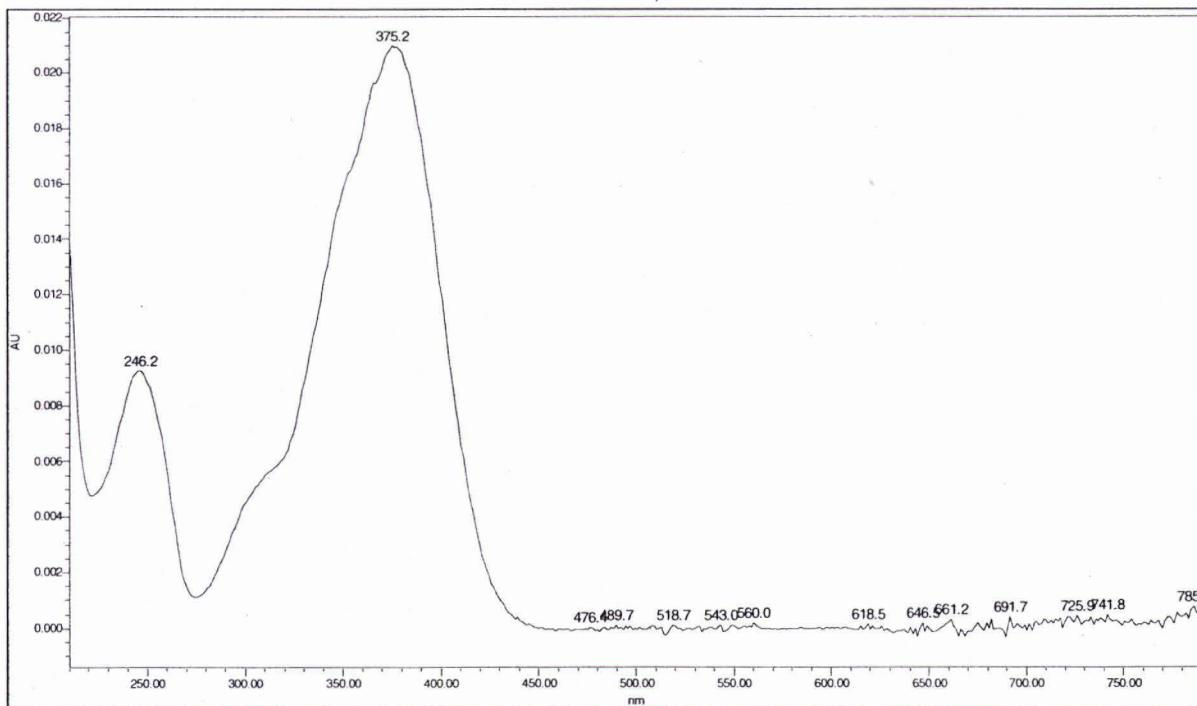
DB14 RT (SHORT GRAD) = 18.7 ± 0.8

DB14 RT (LONG GRAD) = 33.9 ± 1.0



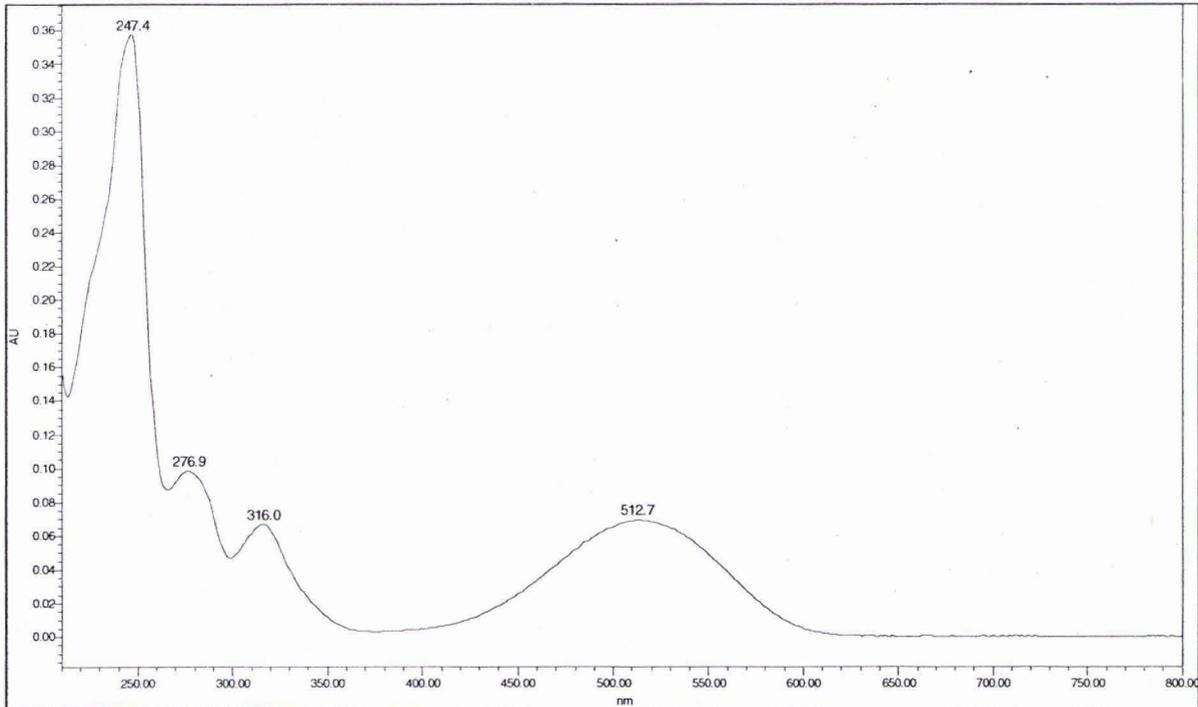
SY3 RT (SHORT GRAD) = 19.8 ± 0.7

SY3 RT (LONG GRAD) = 36.1 ± 1.0



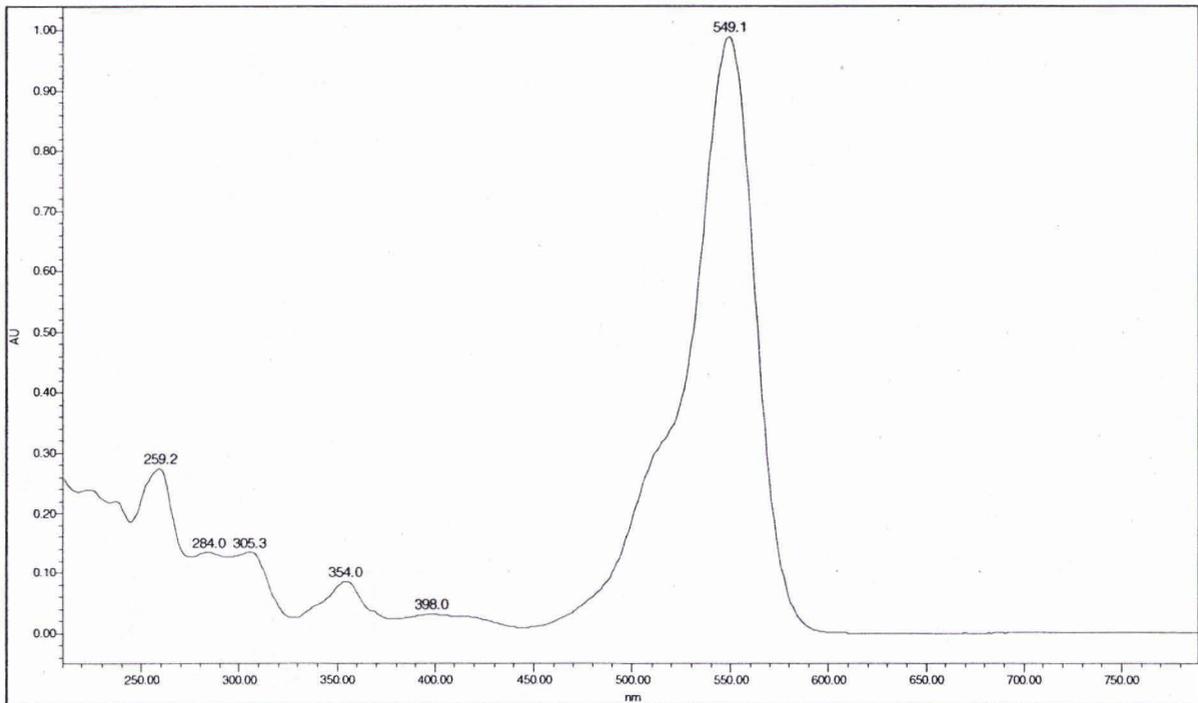
BY2 RT (SHORT GRAD) = 19.3 ± 0.9

BY2 RT (LONG GRAD) = 35.2 ± 1.0



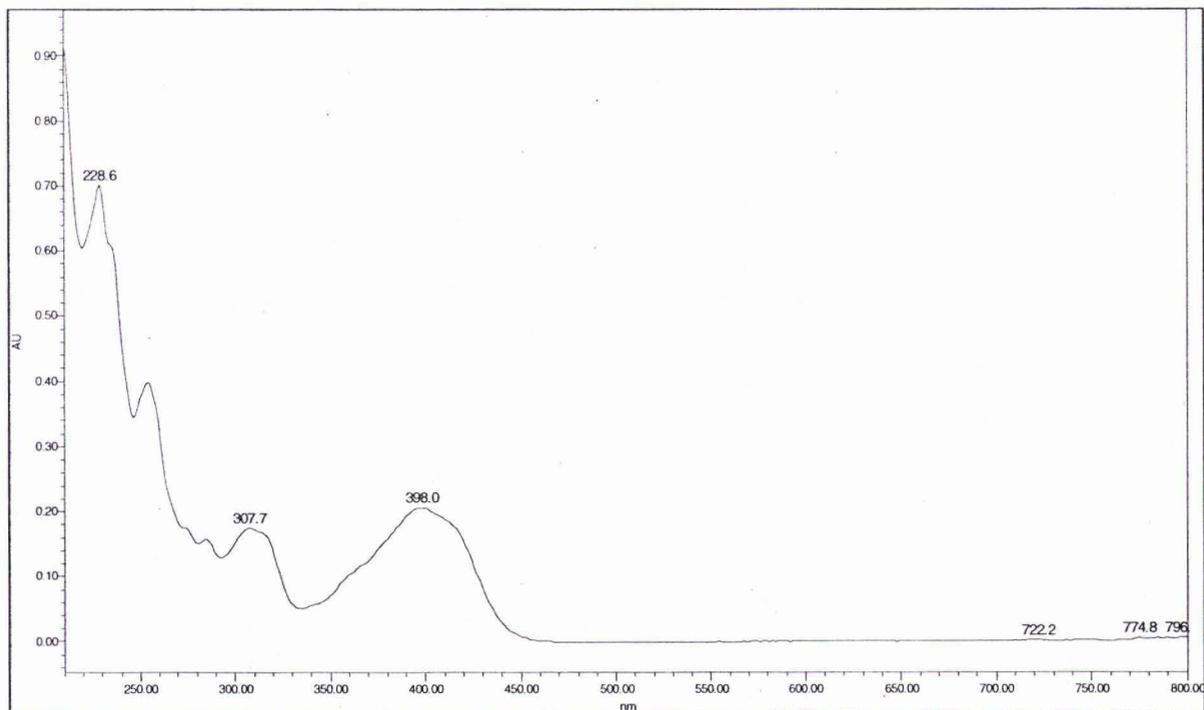
DR9 RT (SHORT GRAD) = 20.2 ± 1.0

DR9 RT (LONG GRAD) = 36.5 ± 1.5



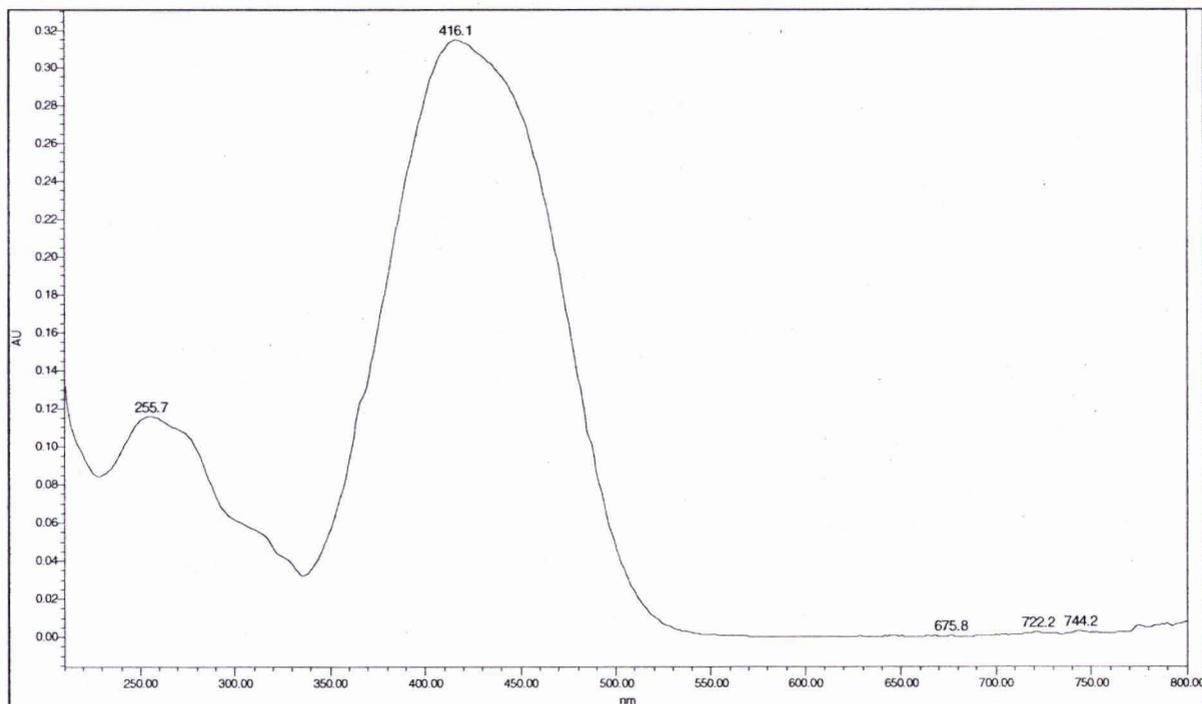
BV10 RT (SHORT GRAD) = 19.6 ± 0.7

BV10 RT (LONG GRAD) = 36.0 ± 0.8



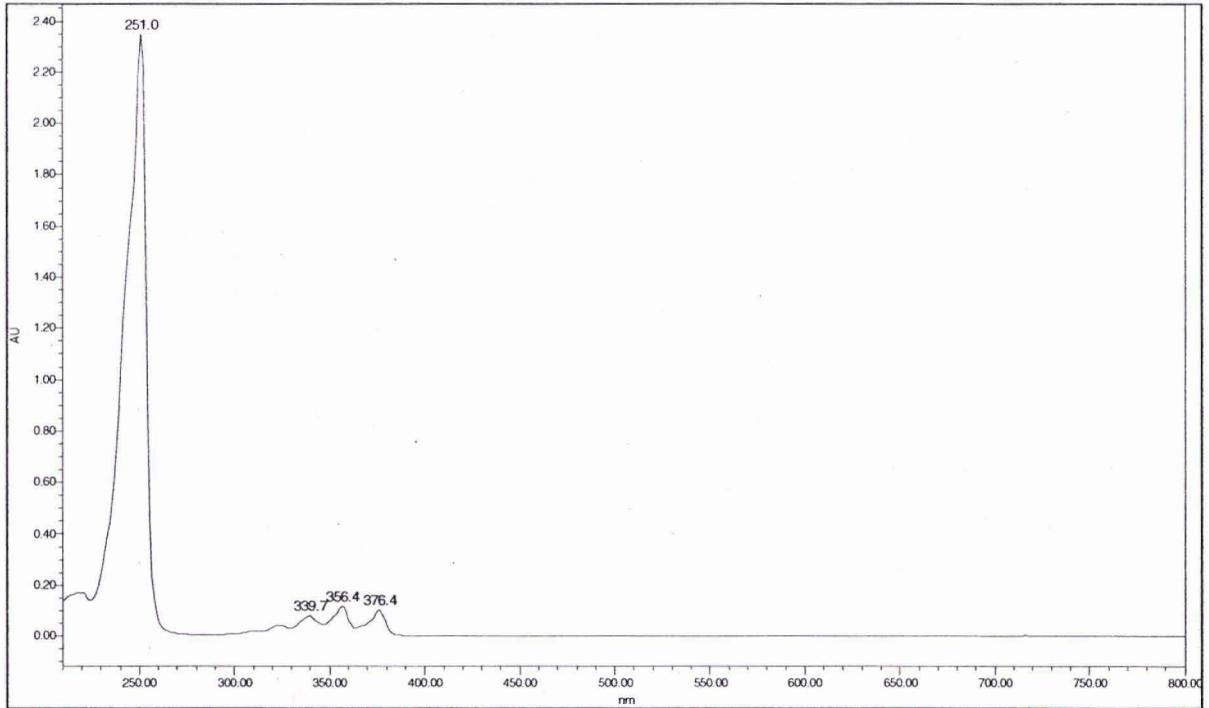
BZ RT (SHORT GRAD) = 20.3 ± 0.7

BZ RT (LONG GRAD) = 36.8 ± 0.9

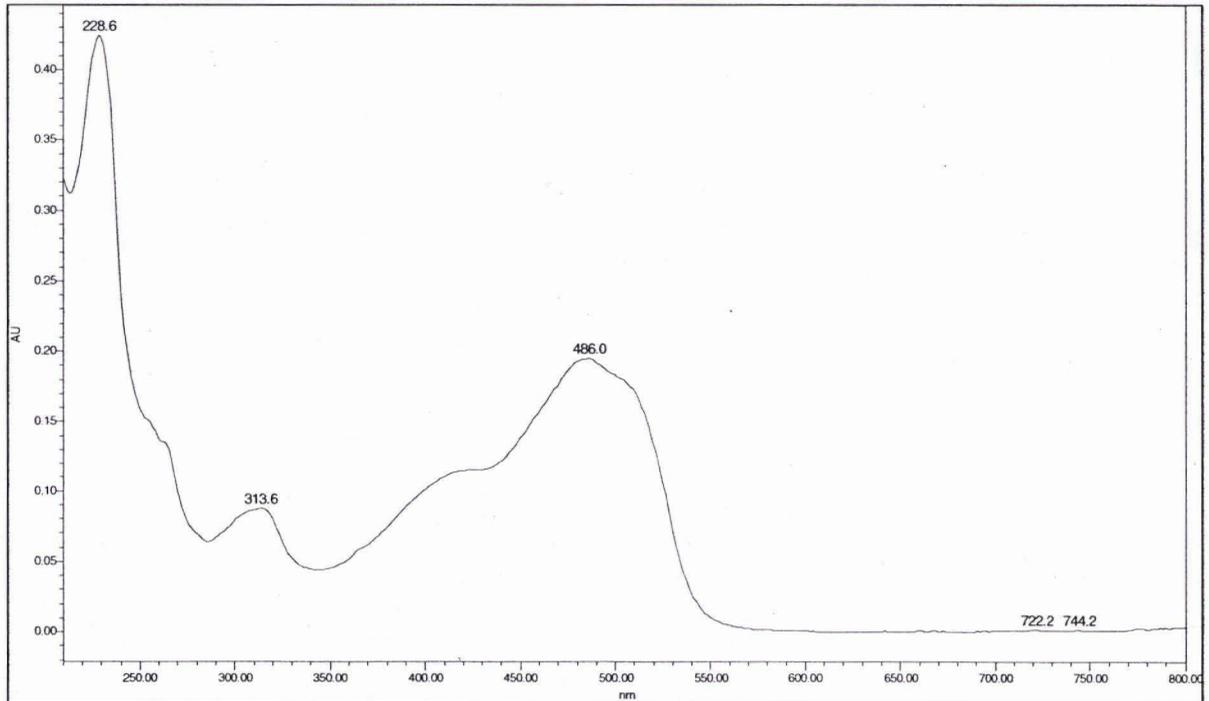


SY2 RT (SHORT GRAD) = 22.1 ± 0.9

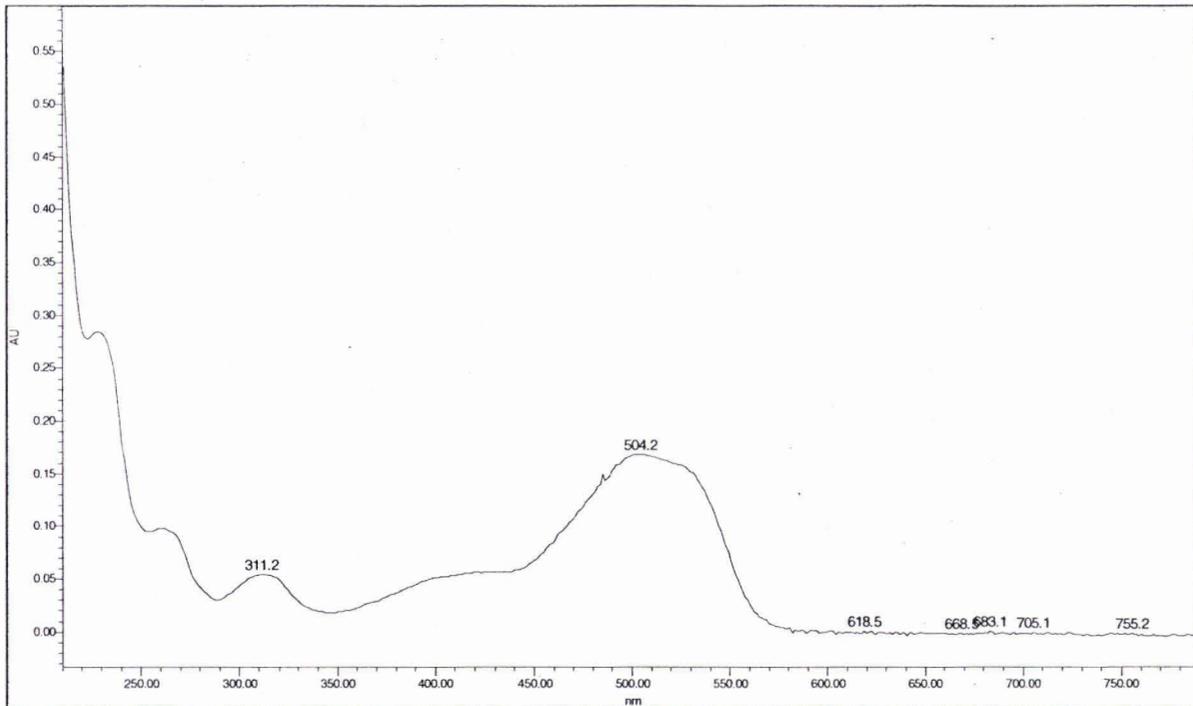
SY2 RT (LONG GRAD) = 40.2 ± 1.4



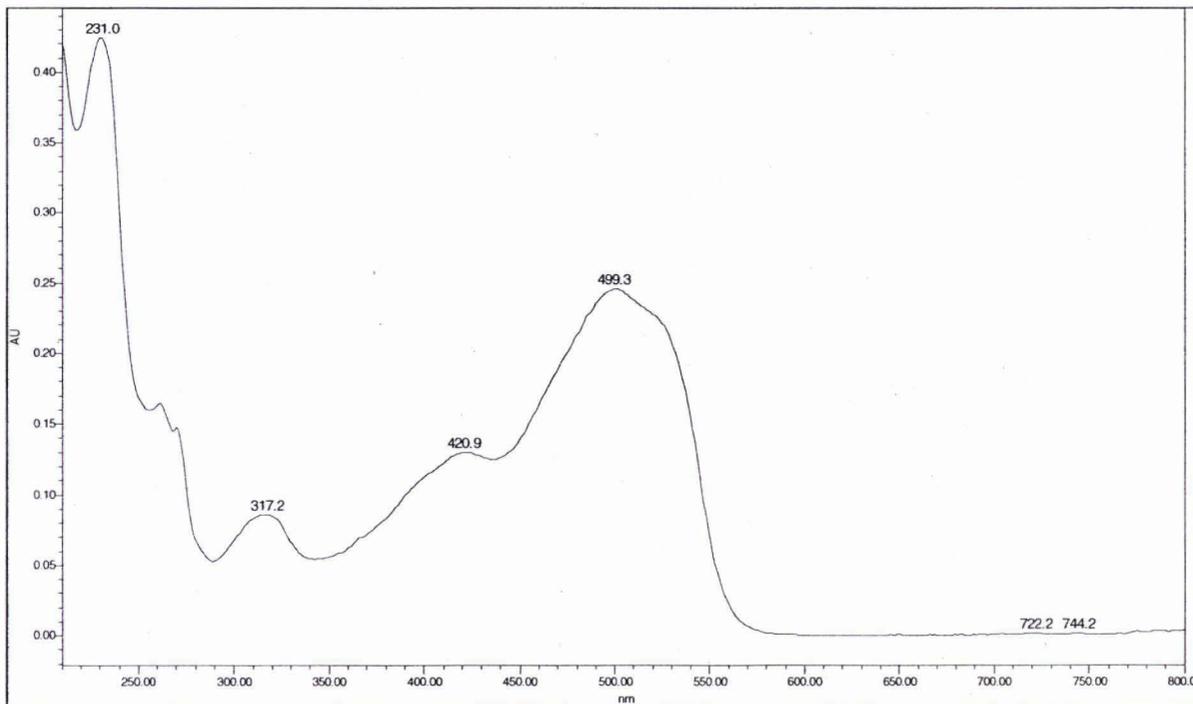
AN RT (SHORT GRAD) = 22.4 ± 0.7
AN RT (LONG GRAD) = 40.7 ± 1.1



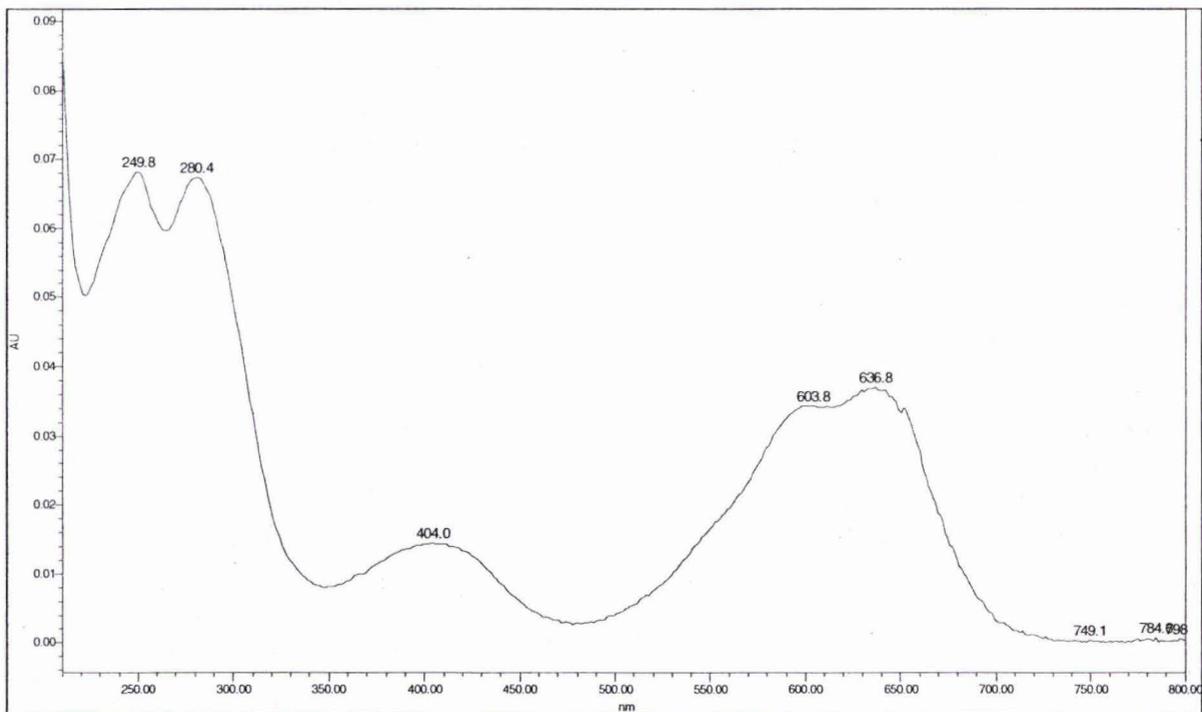
SY14 RT (SHORT GRAD) = 24.3 ± 0.7
SY14 RT (LONG GRAD) = 44.4 ± 1.3



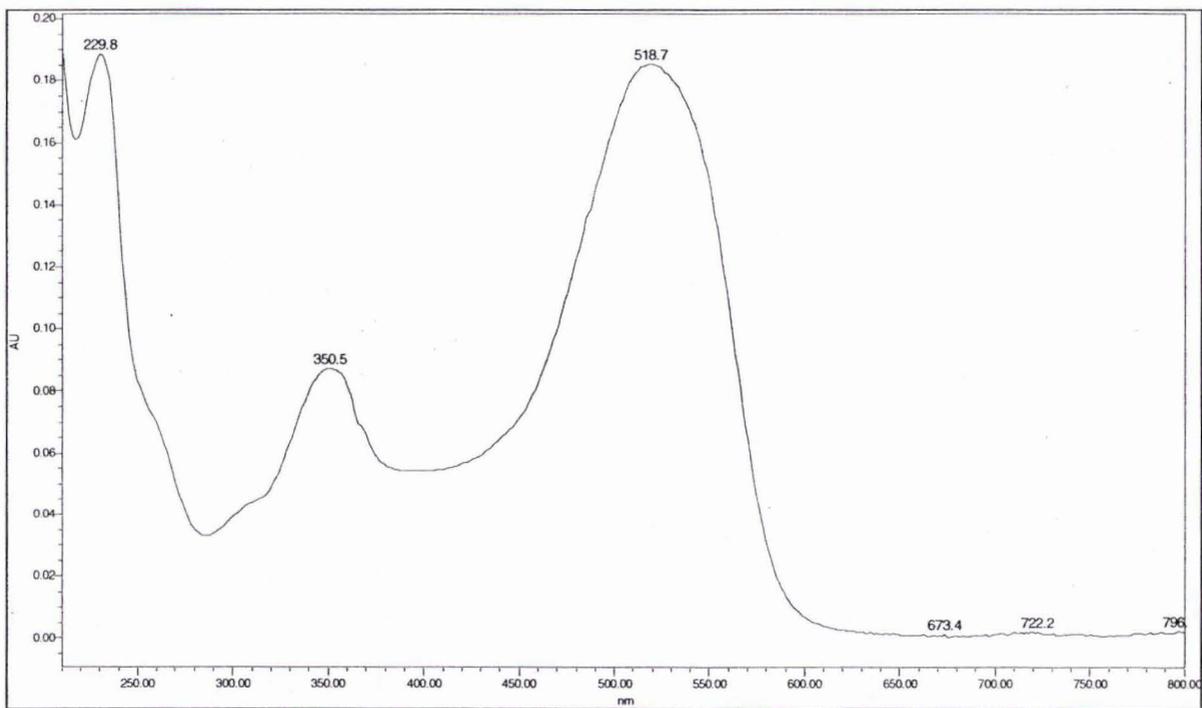
SR1 RT (SHORT GRAD) = 24.5 ± 0.8
SR1 RT (LONG GRAD) = 44.9 ± 1.1



SO7 RT (SHORT GRAD) = 27.7 ± 0.6
SO7 RT (LONG GRAD) = 50.9 ± 1.2



SG3 RT (SHORT GRAD) = 31.0 ± 0.7
SG3 RT (LONG GRAD) = 57.8 ± 1.3



SR24 RT (SHORT GRAD) = 31.3 ± 0.6
SR24 RT (LONG GRAD) = 58.2 ± 1.0