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# Environmental Technology Verification Report

DAKOTA TECHNOLOGIES, INC.  
BALLAST WATER EXCHANGE ASSURANCE METER  
(BEAM)100

Prepared by  
Battelle

**Battelle**  
*The Business of Innovation*

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 **EPA** U.S. Environmental Protection Agency

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# **Environmental Technology Verification Report**

ETV Advanced Monitoring Systems Center

**DAKOTA TECHNOLOGIES, INC.**  
**BALLAST WATER EXCHANGE ASSURANCE METER**  
**(BEAM) 100**

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## **Notice**

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, has financially supported and collaborated in the extramural program described here. This document has been peer reviewed by the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use.

## Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permittees, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six environmental technology centers. Information about each of these centers can be found on the Internet at <http://www.epa.gov/etv/>.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at <http://www.epa.gov/etv/centers/center1.html>.

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## List of Abbreviations

AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
BEAM	Ballast Water Exchange Assurance Meter
BWE	ballast water exchange
°C	degrees Celsius
CDOM	colored dissolved organic matter
COC	chain of custody
EEM	excitation-emission matrix
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
F	intensity at 460 nm
FWHM	full width at half maximum
HPLC	high-performance liquid chromatography
I <sub>M</sub>	maximum intensity
LRB	laboratory record book
MDL	method detection limit
mL	milliliter
NERL	National Exposure Research Laboratory
nm	nanometer
PC	personal computer
PD	percent difference
PE	performance evaluation
ppb	parts per billion
PT	performance test
QA	quality assurance
QC	quality control
QCS	quality control sample
QMP	quality management plan
R	intensity at 430 nm
R <sup>2</sup>	coefficient of determination
RPD	relative percent difference
RSD	relative standard deviation
SR	Suwannee River
TSA	technical systems audit
UV	ultraviolet

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## **Chapter 1 Background**

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permittees; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The EPA's National Exposure Research Laboratory (NERL) and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of Dakota Technologies, Inc.'s Ballast Water Exchange Assurance Meter (BEAM) 100 in measuring colored dissolved organic matter (CDOM) fluorescence as a tool for evaluating ballast water exchange (BWE).

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## Chapter 2 Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This report provides results for the verification testing of Dakota Technologies, Inc.'s BEAM 100. The following is a description of the BEAM 100, based on information provided by the vendor. The information provided below was not verified in this test.

The BEAM 100 (Figure 2-1) is a portable, handheld fluorimeter designed to generate a response relative to the amount of CDOM in ballast water. The CDOM related response is determined by exciting the sample with near ultraviolet (UV) light and measuring the resulting fluorescence to Raman scatter ratio.

The unit consists of a cuvette well permanently mounted in the BEAM. The BEAM is operated through four user-interface buttons. Acquired data are shown in a display screen and can be transferred to a personal computer (PC) for long-term storage. Internally, the BEAM consists of electronics; a light-emitting diode used as an excitation source; and two photodetectors, each with different wavelength filters. All measurements are recorded to the BEAM's internal memory. The BEAM's durable plastic carrying case includes space for cuvette cleaning and sample filtering accessories. The BEAM unit is 10.5 by 4.5 by 3.0 inches and weighs 2.5 pounds (with batteries). The carrying case is 16 by 12 by 7 inches and weighs approximately 10 pounds with the BEAM unit and kit supplies in place. The BEAM 100 costs approximately \$6,000 per unit.



**Figure 2-1. Dakota Technologies, Inc.'s Ballast Water Exchange Assurance Meter (BEAM) 100**

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## Chapter 3

### Test Design and Procedures

#### 3.1 Introduction

Mid-ocean ballast water exchange (BWE) is mandatory for all vessels entering U.S. waters from outside the 200-mile exclusive economic zone. To support such regulation, accurate and portable verification tools are needed for determining that BWE has taken place. One parameter proposed as a means of distinguishing between coastal and open ocean water content in ballast water is fluorescence due to colored dissolved organic matter (CDOM).<sup>(1,2,3)</sup> CDOM refers to the fraction of dissolved organic matter that absorbs light and fluoresces in the UV and visible regions of the spectrum.

The objective of this verification test was to evaluate the performance of the BEAM 100 in measuring CDOM relative to a standard CDOM measurement approach using a laboratory bench-scale excitation-emission spectrometer (Varian Cary Eclipse spectrometer) under controlled laboratory conditions. This verification test was conducted from March to April 2007 according to procedures specified in the *Test/QA Plan for Verification of Ballast Water Exchange Screening Tools* including Amendments 1 and 2.<sup>(4)</sup> This evaluation assessed the capabilities of the BEAM 100 in both laboratory-prepared, performance test (PT) samples and real-world open-ocean and coastal environmental samples. This test did not verify that the BEAM 100 successfully quantified CDOM concentrations or detected BWE, but rather evaluated how well it measured fluorescence from CDOM compared with a standard technique for measuring fluorescence. This test also did not represent all types of waters that may be encountered in ballast water screening, but a range of water (and subsequently the range of fluorescence measurements generated from various types of water) that may be expected in practical application.

The BEAM 100 was verified by evaluating the following parameters:

- Accuracy—Comparison of the BEAM 100 CDOM measurement to CDOM measurements generated by a Varian Cary Eclipse spectrometer with both instruments at ambient laboratory temperature (approximately 24°C).
- Linearity—CDOM measurements from varying concentrations of standard analytes known to fluoresce plotted against the analyte concentration. Linearity was evaluated based on linear regression statistics (i.e., slope and correlation coefficients).
- Precision—The relative standard deviation (RSD) of replicate measurements of the same sample.

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- Method detection limit (MDL)—Analysis of seven replicates of known fluorescing analytes at a concentration five times Dakota Technologies, Inc.’s expected detection limit for the analyte.
  - Inter-unit reproducibility—Relative percent difference (RPD) between the average of triplicate CDOM measurements of the same sample taken at the same temperature using two different BEAM 100 units.
  - Temperature effects—Comparison of the BEAM 100 CDOM measurements at approximately 4 degrees Celsius (°C) and 34°C with CDOM measurements at ambient laboratory temperature (approximately 24°C).
  - Matrix effects—Evaluated by comparing the percent difference (PD) of the BEAM 100 measurements with the Varian Cary Eclipse spectrometer measurements for the various types of samples analyzed during verification testing.
  - Data completeness—The number of valid measurements out of the total number of measurements taken.
  - Operational factors—Observations and records related to maintenance needs, calibration frequency, data output, consumables used, ease of use, repair requirements, waste production, and sample throughput.

### **3.2 Test Facility**

Laboratory analyses of the BEAM 100 were conducted in Battelle laboratories in Columbus, Ohio. No field portability testing was conducted during this technology verification, although temperature and matrices evaluated were varied to simulate field conditions.

### **3.3 Test Procedures**

Test samples used in the verification test included performance test (PT) samples, environmental samples, and quality control (QC) samples as summarized in Table 3-1. These various types of samples are discussed in Section 3.3.1, with the exception of the QC samples, which are described in Section 4.1. The PT and environmental samples were analyzed in triplicate and compared with triplicate measurements taken with the reference method. These samples were evaluated for accuracy compared with expected measurements based on the reference method CDOM analyses, instrument linearity across the range of concentrations tested, and precision among the replicate measurements obtained. Two BEAM 100 units were used to measure the test samples. Measurements of aliquots of the same sample were taken sequentially with the two units and with the reference method within minutes of each other. Inter-unit reproducibility was evaluated based on the measurements taken with the two BEAM units. All measurements made for direct comparison with the reference method were conducted at ambient room temperature. The reference method spectrometer, a Varian Cary Eclipse spectrometer, was configured to be as similar to the BEAM units as possible. Bandwidths were set the same as the BEAM at 10 nm full width at half maximum (FWHM) for all comparison tests, cell geometry was positioned with 90 degrees between the excitation source and the emission detector, and a 1 cm path length cuvette was used. During testing, temperatures were monitored by recording an air reading, a water

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reading, and the BEAM unit internal temperature reading. Ambient room temperatures observed during testing were as follows: 19.3 to 24.4°C for air, 19.6 to 21.4°C for water, and 22 to 28°C for BEAM internal measurements. Although the temperature varied slightly during the course of testing, the ambient room temperature tests will be referred to as 24°C tests in this report. Also note that while the BEAM internal temperature was consistently slightly higher than either the air or water temperature readings, the instruments and test solutions were sufficiently equilibrated that adding sample to the BEAM cell did not change the BEAM internal temperature and the BEAM internal temperature remained constant during all measurements.

Because these technologies will be used in a wide range of temperatures in practical application and because temperature can affect CDOM fluorescence, a subset of test samples was analyzed using only the BEAM 100 units at two additional temperatures (approximately 4°C and 34°C) to evaluate the BEAM 100's variability due to temperature effects. Testing at 4°C took place inside a walk-in refrigerator and testing at 34°C took place inside a chamber where the elevated temperature was created using space heaters and heat lamps. Actual temperatures measured during the temperature extreme testing at 4°C were 4.8 to 7.9°C for air, 6.1 to 8.3°C for water, and 9 to 13°C for BEAM internal measurements. For testing at 34°C, the actual temperatures measured were 33.4 to 35.2°C for air, 34.3 to 35.3°C for water, and 36 to 39°C for BEAM internal measurements. Although the temperature varied during the temperature extreme tests, as also during the ambient room temperature testing, these tests will be referred to as 4°C and 34°C tests in this report. Note that while temperature is one of several variables that might affect practical application (other possibilities include humidity, ambient light, and exposure to the elements), this verification test evaluated only the effect of varying temperature (one temperature above and one temperature below ambient laboratory temperature) on the BEAM 100's performance.

The procedures for preparing, storing, and analyzing test samples are provided below.

### ***3.3.1 Test Sample Collection and Preparation***

#### **3.3.1.1 Performance Test (PT) Samples**

PT samples were created by adding compounds known to cause fluorescence (i.e., quinine sulfate and SR fulvic acid) at multiple concentration levels to Burdick and Jackson HPLC grade water. Burdick and Jackson HPLC grade water was selected because (1) it is certified as having low levels of organic compounds and (2) it was checked for interferences in the wavelengths of interest for verification testing [430 and 460 nanometers (nm)] and found to be clean, with a ratio of 460 nm/430 nm of approximately 0.02. The quinine sulfate samples were prepared in 0.1M sulfuric acid solution, which was made with EMD Chemicals Inc. GR ACS grade sulfuric acid and the Burdick and Jackson HPLC grade water following ASTM E579-04,<sup>(5)</sup> including evaluating the 0.1M sulfuric acid solution for fluorescence prior to use and using the 0.1M solution as the unspiked blank sample for the quinine sulfate samples. Fulvic acid samples were prepared in Burdick and Jackson HPLC grade water with no acidification. The fulvic acid solutions were swirled and allowed to dissolve until no visible particles remained. The stock solution and any subsequent dilutions of the stock were visibly checked to be free of precipitation before their use. Because the fulvic acid solutions were to be prepared in water only, with no acidification as a

**Table 3-1. Verification Test Samples**

	Performance Factor	Sample Description	Replicates for Each BEAM 100 unit		
			4°C	24°C	34°C
<b>PT Samples</b>					
Quinine sulfate prepared in Burdick and Jackson HPLC <sup>a</sup> grade water per ASTM <sup>b</sup> E579-04 <sup>(5)</sup>	Accuracy, linearity, precision, temperature effects	Unspiked	3	3	3
		1 ppb <sup>c</sup> quinine sulfate	3	3	3
		5 ppb quinine sulfate	3	3	3
		10 ppb quinine sulfate	3	3	3
		50 ppb quinine sulfate	3	3	3
		100 ppb quinine sulfate	3	3	3
	MDL	Quinine sulfate at 1 ppb (5 x Dakota-provided detection limit of 0.2 ppb)	-	7	-
Fulvic acid prepared in Burdick and Jackson HPLC grade water	Accuracy, linearity, precision, temperature effects	Unspiked	3	3	3
		100 ppb SR <sup>d</sup> fulvic acid	3	3	3
		500 ppb SR fulvic acid	3	3	3
		1,000 ppb SR fulvic acid	3	3	3
		5,000 ppb SR fulvic acid	3	3	3
		10,000 ppb SR fulvic acid	3	3	3
	MDL	SR fulvic acid at 100 ppb (5 x Dakota-provided detection limit of 20 ppb)	-	7	-
<b>Environmental Samples</b>					
Location 1-open ocean	Matrix effects	Unspiked	-	3	-
Location 2-open ocean			-	3	-
Location 3-coastal			-	3	-
Location 4-coastal			-	3	-
Location 5-coastal			-	3	-
Location 6-coastal			-	3	-
Location 7-coastal			-	3	-
Location 8-coastal			-	3	-
Location 9-coastal			-	3	-
Location 10-coastal			-	3	-
Location 11-coastal			-	3	-
Location 12-coastal			-	3	-
<b>QC Samples</b>					
Negative control	N/A <sup>e</sup>	Burdick and Jackson HPLC grade water	19		
Positive control	N/A	5,000 ppb SR fulvic acid	11		
Calibration check	N/A	10 ppb quinine sulfate	Single measurement minimally every nine verification sample measurements. A total of 24 calibration check measurements were made.		
<b>TOTAL</b>			<b>212</b>		

Shading indicates samples that were also analyzed using the reference method.

a: HPLC = high-performance liquid chromatography

b: ASTM = American Society for Testing and Materials

c: ppb = parts per billion

d: SR = Suwannee River

e: N/A = not applicable, as QC samples were used to monitor BEAM and reference method performance during verification testing.

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preservative, recording pH of the fulvic acid solutions was included in the test/QA plan<sup>(4)</sup> to ensure that the fulvic acid solutions did not change over time. This pH measurement step was inadvertently omitted during testing and is a deviation from the test/QA plan protocol. However, during testing, the fulvic acid solutions were prepared fresh from a stock solution each testing day and testing was completed within a 12-hour period. Because the fulvic acid solutions were made new from the stock solution every day of testing, were not subject to long periods of time before use, and were analyzed sequentially by the BEAM 100 and the reference method within minutes of each other, there was no need to monitor the pH on a continual basis; thus, the absence of solution pH measurements did not have any negative impact on the test results. The fulvic acid solutions were prepared at a concentration 100 times that of the quinine sulfate solutions because, on an equivalent weight basis, fulvic acid produces a much lower fluorescence yield compared to quinine sulfate.

### 3.3.1.2 Environmental Samples

Many sources can contribute to CDOM in a sample.<sup>(1)</sup> These sources can vary from location to location and at various times within the same location can contain large differences in fluorescing materials. Environmental samples were included in verification testing to simulate real samples that may be found in practical application of BWE screening and that would have more complex fluorescence patterns than a simple standard such as quinine sulfate. A total of 15 environmental samples were obtained, consisting of 13 coastal water samples that were collected from areas around the United States between October 2006 and February 2007 and two open ocean samples that were purchased standard reference materials (NASS-5 and MOOS-1) available from National Research Council Canada (Ottawa, Ontario, Canada).

Prior to verification testing, the environmental samples from all 15 locations were screened for their CDOM response using the reference method instrumentation to select a subset of these samples for inclusion in testing. Table 3-2 lists locations where environmental samples were collected and the CDOM screening value obtained for each location. Full excitation-emission matrix (EEM) measurements using the reference method instrumentation were also obtained on all environmental samples to provide additional spectroscopic information that may not be revealed by a single emission scan to aid in selecting a subset to include in testing. EEMs are valuable in analyzing seawater samples because of the many variables that can be included in the analysis. These observations were made to remove any possible outliers such as those that might be produced by observing samples contaminated from an oil slick or some other event in the collection process. The aim was to choose a set of environmental samples that would span the typical fluorescence patterns in “real-world” seawater samples. Because these EEMs were only used to screen samples for use in testing, the excitation intervals were set wider (25-nm intervals) than might be typical if detailed EEMs were needed (5-nm).

Figures 3-1 to 3-5 present three-dimensional (3-D) and contour EEM plots of excitation vs. emission vs. intensity for five of the environmental samples collected for this test and are typical of the EEMs observed overall. Labels of X, R, and F found in each image are the three wavelengths used by the reference method and the BEAM 100 units in verification testing (excitation X = 375 nm, emission R = 430 nm, and emission F = 460 nm). The EEM data were obtained using a Varian Cary Eclipse spectrometer with an automated collection routine in which the excitations were set between 300 and 450 nm at 25-nm intervals and emission observations were measured between 350 and 550 nm at 5-nm intervals. The excitation wavelength was set first, and then a series of



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emission wavelengths were observed. Each series was stored electronically as an emission scan. The light intensity at each data collection point in the scan results from fluorescence, Raman emission and Rayleigh scatter. The fluorescence is emitted from chromophores in the sample (e.g., CDOM). The Raman peak, which is observed at a wavelength shifted  $3600\text{ cm}^{-1}$  (wavenumbers) from the excitation energy, is attributed to the water matrix. The Rayleigh scatter occurs because all molecules have a cross section to the excitation energy that scatters 90 degrees into the emission spectrometer.

The series of emission scans were combined by Grams 3-D software, which allows orientation for optimum views. The Rayleigh scatter is seen as the series of pyramids toward the back of the images in the 3-D plots in Figures 3-1 to 3-5. The Raman bands are also seen as pyramids (where fluorescence does not overwhelm their intensity), but these pyramids are less intense and are shifted a bit more towards longer wavelengths (e.g. towards red light) compared to the Rayleigh scatter pyramids. The light intensities on the contour plots shown in Figures 3-1 to 3-5 are divided into 19 equal bands ranging from 0 to the full scale intensity as plotted.  $I_M$  is the maximum fluorescence observed outside of the Raman or excitation signal regions (i.e., away from the regions where the Rayleigh scatter and Raman pyramids are observed) and varied with each sample. The maximum fluorescence intensity,  $I_M$ , for each sample is listed in the figure captions. In principle, the excitation and Raman signals could be subtracted to obtain a plot showing a maximum resulting only from CDOM fluorescence. However, for sample screening purposes this was not necessary.

The EEM data for each environmental sample were reviewed. All samples appeared to be free of extraneous contamination, with the exception of the NASS-5 open-ocean seawater standard shown in Figure 3-4. The NASS-5 open-ocean seawater standard had more fluorescence at excitations of 300 nm and 325 nm with emission in the 450-nm spectral region than would be expected, based on experience and other clean samples. Three of the other four samples shown in the figures above have  $I_M$  values less than the  $I_M$  of the NASS-5 sample even though they are coastal waters. It was noted that the NASS-5 standard was packed in a plastic container. Plastic containers are known to leach compounds that fluoresce in the low 400-nm region when excited in the UV region of the spectrum, and this is a likely cause of the observed fluorescence. However, at 375-nm excitation, which is the excitation wavelength used in the verification test, the emission intensities were unchanged from the other clean waters and, as a result, the NASS-5 open-ocean seawater standard was used in testing since the fluorescence, possibly due to the plastic container, would not affect the comparison of the reference method measurements with BEAM 100 measurements.

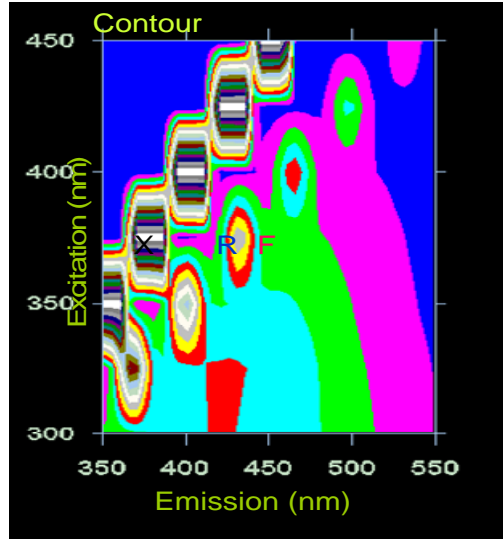
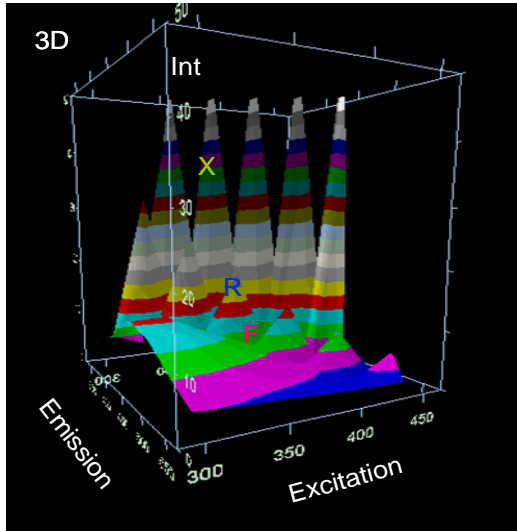
Based on the EEMs and the screening CDOM responses, 12 of the 15 environmental samples were selected for inclusion in the verification test. The environmental samples included in the verification test are noted in Table 3-2.

**Table 3-2. Environmental Samples**

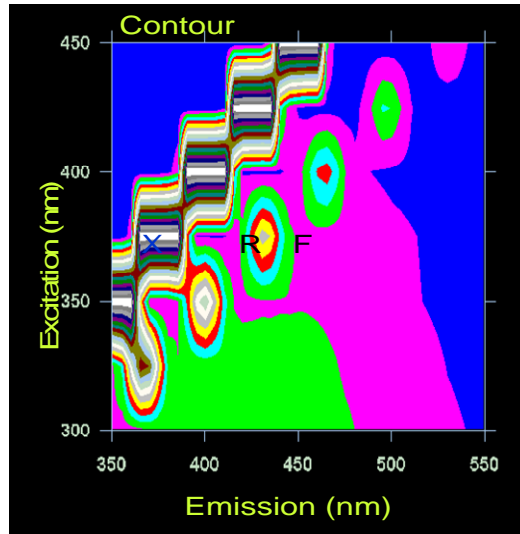
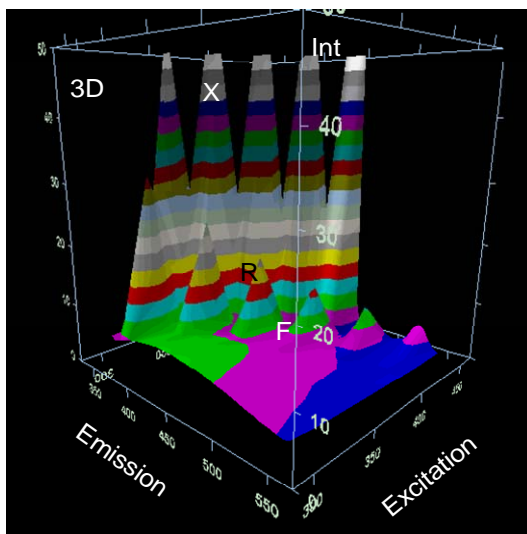
<b>Location</b>	<b>Description</b>	<b>Sample Type</b>	<b>Collection Date</b>	<b>Storage Conditions</b>	<b>Screening CDOM Ratio</b>	<b>Used in Testing</b>
Duxbury Bay, MA	Off the dock at Battelle Duxbury Operations in Duxbury, MA	Coastal seawater	1/24/2007	Unfiltered, stored cool (~4 °C) after collection.	0.47	No
Boston Harbor, MA	Inside of Neponset Estuary in Boston Harbor, MA	A mixture of freshwater and coastal seawater with expected high CDOM fluorescence	11/10/2006	Filtered with a 0.7- $\mu$ m glass fiber filter upon return to the laboratory. Frozen until shipped to Battelle. Stored cool (~4 °C) after receipt at Battelle.	1.40	Yes
Massachusetts Bay NF7, MA	Nine miles east of Deer Island, MA	Coastal seawater	11/18/2006	Filtered in the field with a 0.7 $\mu$ m glass fiber filter, frozen after collection until shipped to Battelle. Stored cool (~4 °C) after receipt at Battelle.	0.43	Yes
Massachusetts Bay NF10, MA	Nine miles east of Deer Island, MA	Coastal seawater	11/18/2006	Filtered in the field with a 0.7 $\mu$ m glass fiber filter, frozen after collection until shipped to Battelle. Stored cool (~4 °C) after receipt at Battelle.	0.44	No
Sequim Bay, WA	Off the dock at Battelle Marine Sciences Lab in Sequim, WA	Coastal seawater	1/30/2007	Unfiltered, stored cool (~4 °C) after collection.	0.33	No
Puget Sound, WA	Outside of Ediz Hook in Port Angeles, WA	Coastal seawater	1/30/2007	Unfiltered, stored cool (~4 °C) after collection.	0.28	Yes
East Coast, FL-1	Inter-coastal water way in West Palm Beach, FL	A mixture of freshwater and coastal seawater with expected high CDOM fluorescence	1/28/2007	Unfiltered, stored cool (~4 °C) after collection.	1.28	Yes
East Coast, FL-2	Atlantic Ocean beach off Palm Beach, FL	Coastal seawater	1/28/2007	Unfiltered, stored cool (~4 °C) after collection.	0.24	Yes

**Table 3-2. Environmental Samples (continued)**

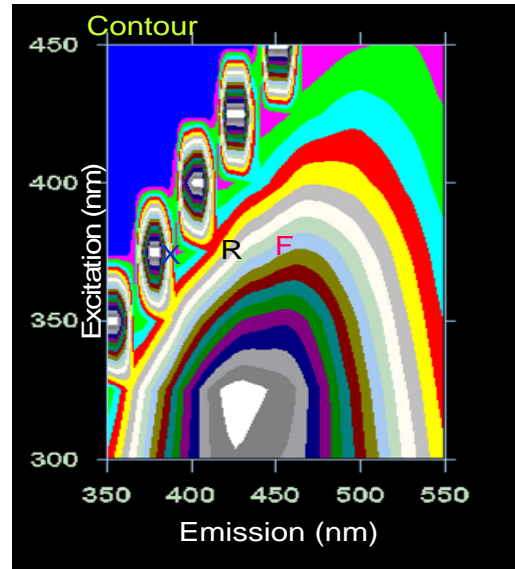
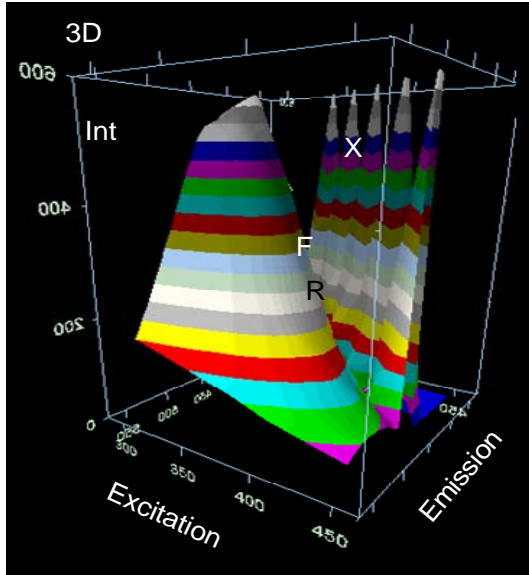
<b>Location</b>	<b>Description</b>	<b>Sample Type</b>	<b>Collection Date</b>	<b>Storage Conditions</b>	<b>Screening CDOM Ratio</b>	<b>Used in Testing</b>
Open Ocean - 1	NASS-5 Open Ocean Seawater Reference Material for Trace Metals collected 35 kilometers southeast of Halifax, NS, Canada	Open ocean seawater with expected low CDOM fluorescence	Prior to June 1998	Filtered through a 0.45 micron filter and then acidified to pH 1.6 with ultrapure nitric acid. Stored cool (~4 °C) after collection.	0.31	Yes
Open Ocean - 2	MOOS-1 Seawater Certified Reference Material for Nutrients collected off the northern tip of Cape Breton Island, NS, Canada	Open ocean seawater with expected low CDOM fluorescence	6/24/1996	Filtered through a 0.05 micron cartridge filter after collection, irradiated after bottling. Stored cool (~4 °C) after collection.	0.20	Yes
Long Island Sound, NY	Dock in Port Jefferson, NY	Coastal seawater	2/4/2007	Unfiltered, stored cool (~4 °C) after collection.	0.63	Yes
New York Harbor, NY	East River, NY	A mixture of freshwater and coastal seawater with expected high CDOM fluorescence	2/4/2007	Unfiltered, stored cool (~4 °C) after collection.	0.85	Yes
New York Bight, NY	Atlantic Ocean sample from a beach in South Hampton, NY	Coastal seawater	2/4/2007	Unfiltered, stored cool (~4 °C) after collection.	0.46	Yes
San Diego Harbor, CA	San Diego Harbor, CA	Coastal seawater	1/22/2007	Unfiltered, stored cool (~4 °C) after collection.	0.37	Yes
Narragansett Bay, RI	Off 2-14 Great Island Rd, Narragansett, RI	Coastal seawater	10/21/2006	Unfiltered, stored cool (~4 °C) after collection.	0.53	Yes



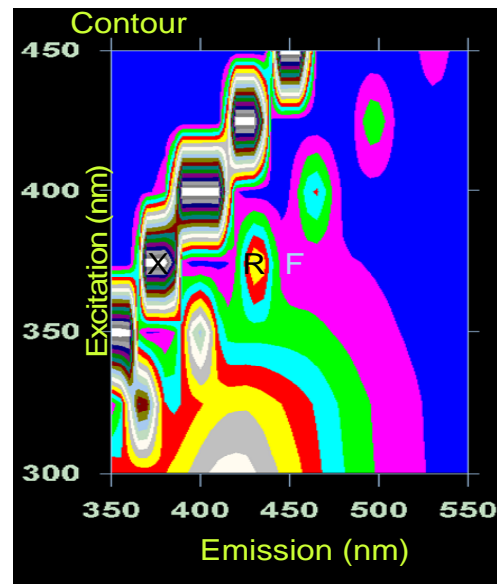
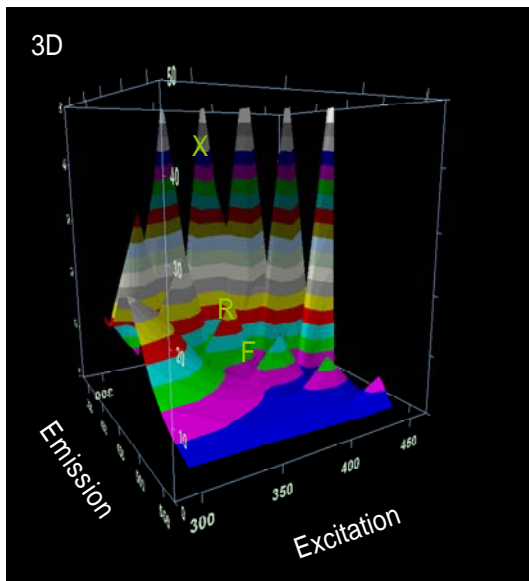
**Figure 3-1. 3-D Plot of Seawater Collected in Sequim Bay, Washington State. Intensity and features are representative of the received seawater samples with a maximum intensity ( $I_M$ ) = 14.**



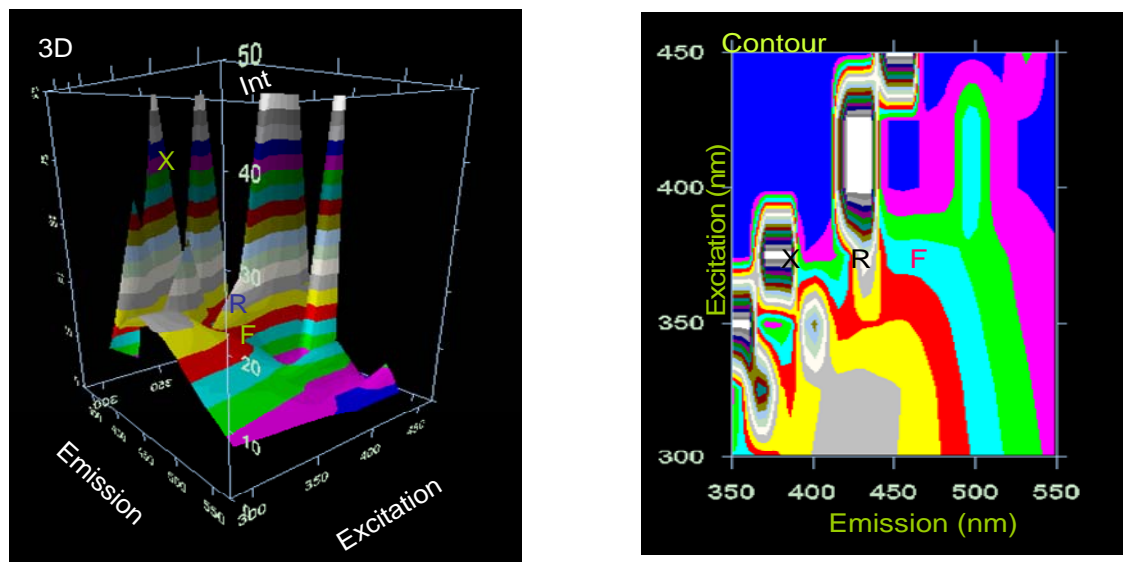
**Figure 3-2. Seawater Collected at a Beach in Palm Beach, Florida. Intensity and features are cleaner than typical seawater samples received with  $I_M = 10$ .**



**Figure 3-3. Seawater Collected in an Intercoastal Waterway in West Palm Beach, Florida. Note the high intensity for this coastal sample where  $I_M = 550$ .**



**Figure 3-4. NASS-5 Standard. Features are representative of the received seawater samples, but  $I_M$  of 20 at excitation 300 nm is high for open-ocean water.**



**Figure 3-5. Seawater from Duxbury Bay, Massachusetts. Intensity and features are representative of the received seawater samples. The  $I_M$  at excitation 300 nm is 17.**

### 3.3.1.3 Quality Control Samples

QC samples are discussed in Section 4.1.

### 3.3.2 Test Sample Analysis Procedure

According to the cleaning and rinsing instructions in the BEAM 100 user manual, each unit was flushed with distilled water, cleaned using the cleaning solution and swabs provided with the unit, and then rinsed with copious amounts of distilled water. Once cleaned and rinsed, the units were blank calibrated using Burdick and Jackson HPLC grade water, which was used to prepare all calibration standards and PT samples used in verification testing. Blank calibration followed the process listed in the instruction manual and was performed at the beginning of testing, prior to quinine sulfate calibrations or any test sample analysis. The units were then ready for use in verification testing. At the start of testing at each of the three temperatures used in verification testing ( 24°C, 4°C, and 34°C), the BEAM 100 units were calibrated with quinine sulfate by allowing the units and standard solution (10 ppb quinine sulfate) to come to the testing procedure operating temperature with at least a 30-minute temperature equilibration time. Each unit was calibrated with 10 ppb quinine sulfate following the “standard-point calibration process” listed in the user manual, which consisted of filtering the calibration solution into the BEAM cell using the syringes and 0.45-micron filters supplied with each unit, tightly capping the cell, and then simultaneously pressing the “CAL” and “RUN” buttons on the BEAM surface. The blank calibration and quinine sulfate standard calibration was repeated only if it was necessary to address a testing malfunction. After calibrating at the appropriate temperature, the units were ready for sample measurement.

After the sample was allowed to equilibrate at the testing temperature for at least 30 minutes, a sample measurement was acquired by first rinsing the unit with distilled water as outlined in the user manual rinsing procedure. The sample was then filtered into the BEAM cell using the syringes

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and 0.45 micron filters which are supplied by the vendor and come with the BEAM kit. The cell was rinsed with the sample, the rinse was discarded, and then the cell was filled with sample for testing. Once filled with sample for testing, the cell cap was tightly closed and sample measurements were taken by pressing the “RUN” button on the BEAM surface. After a few seconds, the measured values were displayed on the BEAM unit and were manually recorded on data sheets to provide a backup to the electronic data storage. After a day of analysis was completed and prior to the next day of testing, the acquired data were downloaded to a PC using the software provided by Dakota Technologies, Inc. and following the instructions listed in the user manual.

To compare measurements of the two BEAM units or the two BEAM units and the reference method, aliquots of the same test sample were filtered into the cell of each instrument (BEAM and reference spectrometer). Once all cells were full and caps tightened as appropriate, each unit’s run procedure was initiated.

When QC sample failures occurred (e.g., the quinine sulfate continuing calibration was outside the 0.41 to 0.45 acceptance criterion or the negative control had a reading >2,000 counts in the 460-nm light channel) or a BEAM unit error occurred, the following corrective action process was followed. First, the unit’s cell well was re-rinsed and the sample was re-measured. Following a second measurement error, the unit was re-cleaned, re-rinsed, and the sample re-measured. Following a third measurement error, the unit was re-rinsed, then calibrated, and the sample re-measured. After a fourth measurement error, the unit was re-rinsed, re-blank calibrated, re-calibrated, and the sample re-measured. If none of these corrective actions helped resolve the problem, Dakota Technologies, Inc. was contacted for technical support.

Two BEAM units were received for testing. The units were identified as 100-R2-08 and 100-R2-04 and are referred to as BEAM 08 and BEAM 04 in this report. BEAM 08 and BEAM 04 were used for all 24°C room temperature testing. During testing, some technical difficulties (described in Section 6.9) were encountered with BEAM 08; subsequently, this unit was replaced by Dakota Technologies, Inc. with a unit identified as 100-R2-03 and is referred to as BEAM 03 in this report. BEAM 03 and BEAM 04 were used for the testing at temperature extremes (4°C and 34°C).

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## **Chapter 4**

### **Quality Assurance/Quality Control**

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center<sup>(6)</sup> and the test/QA plan for this verification test,<sup>(4)</sup> except for the deviation discussed in Section 3.3.1.1. QA/QC procedures and results are described below.

#### **4.1 Quality Control Samples**

Steps were taken to maintain the quality of data collected during this verification test. This included analyzing specific quality control samples (QCSs) at a regular frequency. QCSs included negative controls, positive controls, and calibration checks.

##### ***4.1.1 Negative Controls***

Burdick and Jackson HPLC grade water was analyzed as a negative control. Negative control samples were used to help ensure that no sources of contamination were introduced in the sample handling and analysis procedures. Dakota Technologies, Inc. indicated that the negative control should provide a reading of <2,000 counts in the 460-nm light channel. If, at any time, the negative control had more than 2,000 counts in the 460-nm light channel, the cell was cleaned and/or the negative control solution and filter were replaced until a reading <2,000 counts was obtained.

##### ***4.1.2 Positive Controls***

Throughout verification testing, positive control samples consisting of a 5,000-ppb SR fulvic acid solution were analyzed to indicate to the operator that the BEAM 100 units were properly detecting a positive response. CDOM ratio values between 0.86 and 0.89 were obtained for readings taken at 24°C. Slightly lower values (0.70 to 0.74) were obtained for the 5,000-ppb SR fulvic acid solution at 34°C, and slightly higher values (0.93 to 1.16) were obtained at 4°C. The variations with changing temperatures were not unexpected because of the influence temperature can have on fluorescence.

##### ***4.1.3 Calibration Checks***

Calibration checks of 10 ppb quinine sulfate were analyzed, at a minimum, after every nine measurements of PT or environmental samples with the BEAM 100 units. The initial BEAM quinine sulfate calibration set the CDOM ratio of a 10-ppb quinine sulfate solution at 0.43. Subsequent calibration checks required that the CDOM ratio for a 10-ppb quinine sulfate solution be between 0.41 and 0.45. If, at any time, the calibration check did not fall within these limits, the cell was cleaned and the calibration check repeated. If the calibration check continued to remain



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outside the 0.41 to 0.45 limits, the affected BEAM unit was recalibrated. Analysis did not proceed until a successful calibration check was obtained.

## **4.2 Audits**

Three types of audits were performed during the verification test: a performance evaluation (PE) audit of the reference method measurements made in this verification test, a technical systems audit (TSA) of the verification test performance, and a data quality audit. Audit procedures are described further below.

### ***4.2.1 Performance Evaluation Audits***

A PE audit was conducted to assess the quality of the reference method measurements made in this verification test. The reference method PE audit was performed by supplying a second quinine sulfate standard solution prepared from a different source of quinine sulfate than that used in verification testing. The PE audit samples were analyzed in the same manner as all other samples, and the analytical results for the PE audit samples were compared with the nominal concentration. The target criterion for this PE audit was agreement of the analytical result within 3% of the nominal concentration. This audit was performed once prior to the start of the test. The second source PE standard was within 1.32% of the nominal value.

### ***4.2.2 Technical Systems Audit***

The Battelle Quality Manager performed one TSA during this verification test to ensure that the verification test was being performed in accordance with the AMS Center QMP,<sup>(6)</sup> the test/QA plan,<sup>(4)</sup> and standard operating procedures. In the TSA, the Battelle Quality Manager reviewed the reference methods used, compared actual test procedures with those specified or referenced in the test/QA plan,<sup>(4)</sup> and reviewed data acquisition and handling procedures. Also in the TSA, the Battelle Quality Manager observed testing, inspected sample chain-of-custody (COC) documentation, and reviewed technology-specific record books. He also checked standard certifications and technology data acquisition procedures and conferred with the technical staff. A TSA report was prepared, including a statement of findings and the actions taken to address those findings. The TSA findings were communicated to technical staff at the time of the audit. The records concerning the TSA are permanently stored with the Battelle Quality Manager.

### ***4.2.3 Data Quality Audit***

At least 10% of the data acquired during the verification test were audited. Battelle's Quality Manager traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

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### **4.3 QA/QC Reporting**

Each audit was documented in accordance with Section 3.3.4 and 3.3.5 of the QMP for the ETV AMS Center.<sup>(6)</sup> Once the audit reports were prepared, the Battelle Verification Test Coordinator ensured that a response was provided for each adverse finding or potential problem and implemented any necessary follow-up corrective action. The Battelle Quality Manager ensured that follow-up corrective action was taken. The results of the TSA were submitted to the EPA.

### **4.4 Data Review**

Records generated in the verification test received a one-over-one review before these records were used to calculate, evaluate, or report verification results. Table 4-1 summarizes the types of data recorded. A Battelle technical staff member involved in the verification test reviewed the data. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed.

**Table 4-1. Summary of Data Recording Process**

<b>Data to Be Recorded</b>	<b>Responsible Party</b>	<b>Where Recorded</b>	<b>How Often Recorded</b>	<b>Disposition of Data</b>
Dates, times, and details of test events, BEAM 100 maintenance, downtime, etc.	Battelle	ETV laboratory record books (LRBs) or data recording forms	Start/end of test procedure, and at each change of a test parameter or change of BEAM 100 status	Used to organize and check test results; manually incorporated in data spreadsheets as necessary
BEAM 100 calibration information	Battelle	ETV LRBs, data recording forms, or electronically	At BEAM 100 calibration or recalibration	Incorporated in verification report as necessary
BEAM 100 readings	Battelle	Either recorded electronically by the BEAM 100 and downloaded to an independent computer or hard copy data printed by the BEAM 100 and taped into the ETV LRB. Also hand entered into ETV LRBs or data recording forms.	Recorded continuously for electronic data and printed after each measurement for hard copy print-outs and recorded manually with each reading	Converted to or manually entered into spreadsheet for statistical analysis and comparisons
Sample collection and reference method analysis procedures, calibrations, QA, etc.	Battelle and others assisting in sample collection	LRBs, COC, or other data recording forms	Throughout sampling and analysis processes	Retained as documentation of sample collection or reference method performance
Reference method results	Battelle	Electronically or manually into ETV LRBs or data recording forms. Where possible at least the same number or a maximum of one number more significant figures as the BEAM 100 result was reported for the reference method.	Every sample or QC analysis	Transferred to spreadsheets for calculation of results, and statistical analysis and comparisons

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## Chapter 5

### Statistical Methods

The statistical methods used to evaluate the quantitative performance factors listed in Section 3.1 are presented in this chapter. Qualitative observations were also used to evaluate verification test data.

#### 5.1 Accuracy

Accuracy was determined by calculating the percent difference (PD) between the average of triplicate CDOM measurements of a sample solution with the BEAM 100 ( $M_1$ ) and the average of triplicate BEAM equivalent CDOM measurement generated by a Varian Cary Eclipse spectrometer ( $M_2$ ). As noted in Section B4 of the test/QA plan,<sup>(4)</sup> the CDOM ratios of the BEAM and reference methods were not expected to be identical due to differences in grating efficiencies (the BEAM uses filters to separate the light into the 430 and 460 nm wavelengths, whereas the Varian Eclipse spectrometer uses gratings) and other conditions that can vary from instrument to instrument. However, there should be a correlation between values obtained by the BEAM and the reference method. This correlation was obtained by comparing the BEAM and Varian Cary Eclipse spectrometer reference CDOM values for common concentration of quinine sulfate solution measurements at 24°C. The regression statistics between the BEAM and Varian Cary Eclipse spectrometer based on analyzing quinine sulfate solutions on both instruments were then used to convert the Varian Cary Eclipse spectrometer CDOM values into BEAM equivalent values for purposes of evaluating accuracy. The measurements were generated at a single temperature (i.e., data from 24°C measurements were used) for both PT and environmental samples using Equation 1. The relationship between the BEAM and Varian Cary Eclipse spectrometer quinine sulfate curves and additional information on how accuracy was determined using the BEAM and the adjusted Varian Cary Eclipse spectrometer reference method CDOM values are discussed further in Section 6.1.

$$PD(\%) = \frac{|M_1 - M_2|}{M_2} \times 100 \quad (1)$$

PD values less than 20% were targeted as an acceptable demonstration of comparability between the two measurements.

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## 5.2 Linearity

Linearity was determined by plotting the CDOM measurements (fluorescence values generated at a single wavelength) while analyzing varying concentrations of analytes known to fluoresce (y-axis) against the analyte concentration (x-axis) and performing linear curve fitting to determine the slope (m) and intercept (b) in Equation 2.

$$y = mx + b \quad (2)$$

Correlation coefficients such as the Pearson's  $r$  values and coefficient of determination ( $R^2$ ) values were calculated. A perfect regression line would have  $R^2$  values equal to 1.

## 5.3 Precision

The standard deviation ( $S$ ) of the results for the replicate analyses of the same sample was calculated as follows:

$$S = \left[ \frac{1}{n-1} \sum_{k=1}^n (M_k - \bar{M})^2 \right]^{1/2} \quad (3)$$

where  $n$  is the number of replicate samples,  $M_k$  is the CDOM measurement for the  $k^{\text{th}}$  sample, and  $\bar{M}$  is the average CDOM measurement of the replicate samples. The BEAM 100 precision for each sample was reported in terms of the relative standard deviation (RSD), which was calculated as follows.

$$RSD(\%) = \left| \frac{S}{\bar{M}} \right| \times 100 \quad (4)$$

RSD values less than 10% were targeted as an acceptable indication of precise measurements.

## 5.4 Method Detection Limit (MDL)

The MDL was determined according to procedures described in Appendix B in Chapter 40 of the Code of Federal Regulations Part 136 (40 CFR 136)<sup>(7)</sup> and assessed from seven replicate analyses of a fortified sample. Fortified samples were generated by adding known fluorescing compounds (quinine sulfate and SR fulvic acid) to Burdick and Jackson HPLC grade water. The target analyte was added at a concentration approximately five times Dakota's stated detection limit. The MDL was calculated using Equation 5:

$$MDL = t \times S \quad (5)$$

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where  $t$  is the Student's value of 3.143 for a 99% confidence level when the degrees of freedom (N-1, where N equals the total number of measurements in the set) equals six, and  $S$  is the standard deviation of the replicate samples.

### 5.5 Inter-unit Reproducibility

Inter-unit reproducibility was determined by evaluating the relative percent difference (RPD) between the average of triplicate measurements for each sample tested using two separate units of the BEAM 100. The equation for RPD, reported in percent, is as follows:

$$RPD(\%) = \frac{|M_1 - M_2|}{M_1 + M_2} \times 200 \quad (6)$$

where  $M_1$  is the average of triplicate measurements made by the first BEAM 100 and  $M_2$  is the average of triplicate measurements made by the second BEAM 100. RPD values less than 20% were targeted as an indication of good agreement between the two units.

### 5.6 Temperature Effects

Temperature effects were determined by measuring the PD (using Equation 1) between the average of triplicate measurements for each sample at 4°C and 34°C using the BEAM instruments ( $M_1$ ) against the average measurements at 24°C using the BEAM instruments ( $M_2$ ).

### 5.7 Matrix Effects

Matrix effects were determined by comparing the PD measurements between the BEAM results and the reference method results for each type of sample used in testing (the two PT sample types: quinine sulfate and fulvic acid, and the environmental samples). The PD measurements are determined as described in Section 5.1. Trends in PD from the reference method were assessed based on sample type.

### 5.8 Data Completeness

Data completeness was calculated as the percentage of the total possible data. Completeness was determined by dividing the number of valid data measurements generated by each BEAM 100 ( $M_{valid}$ ) by the total number of data measurements included in verification testing ( $M_{total}$ ).

$$Completeness(\%) = \frac{M_{valid}}{M_{total}} \times 100 \quad (7)$$

The cause of any substantial loss of data was established from operator observations or BEAM 100 records and noted in the discussion of the data completeness results.

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## **5.9 Operational Factors**

There were no statistical calculations applicable to operational factors. Operational factors were determined based on documented observations of the testing staff and the Verification Test Coordinator.

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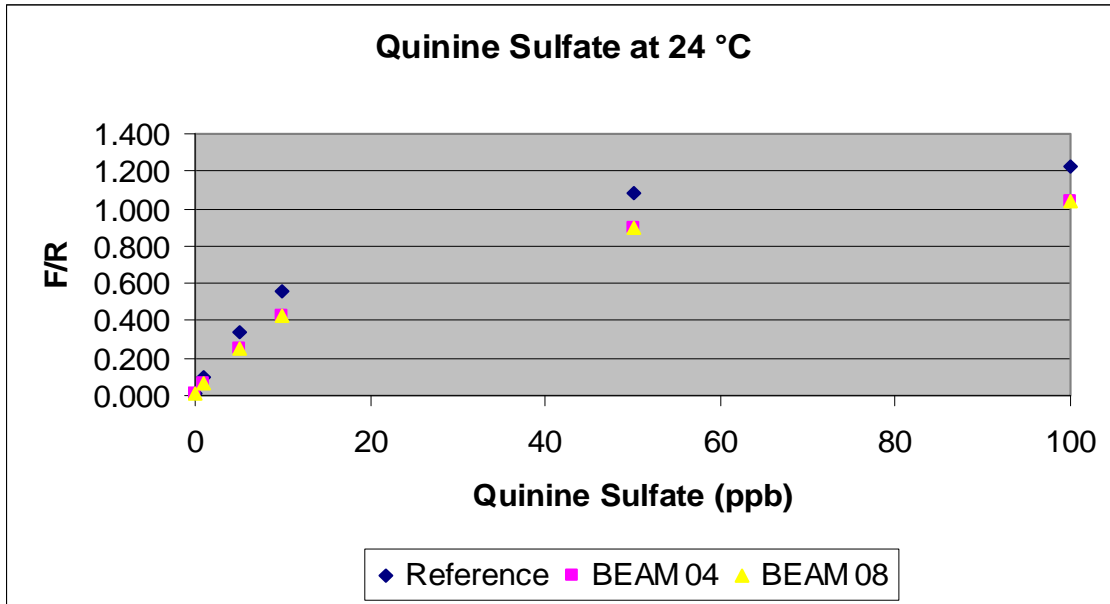
## Chapter 6 Test Results

The results of the verification tests of the BEAM 100 are presented below for each of the performance parameters.

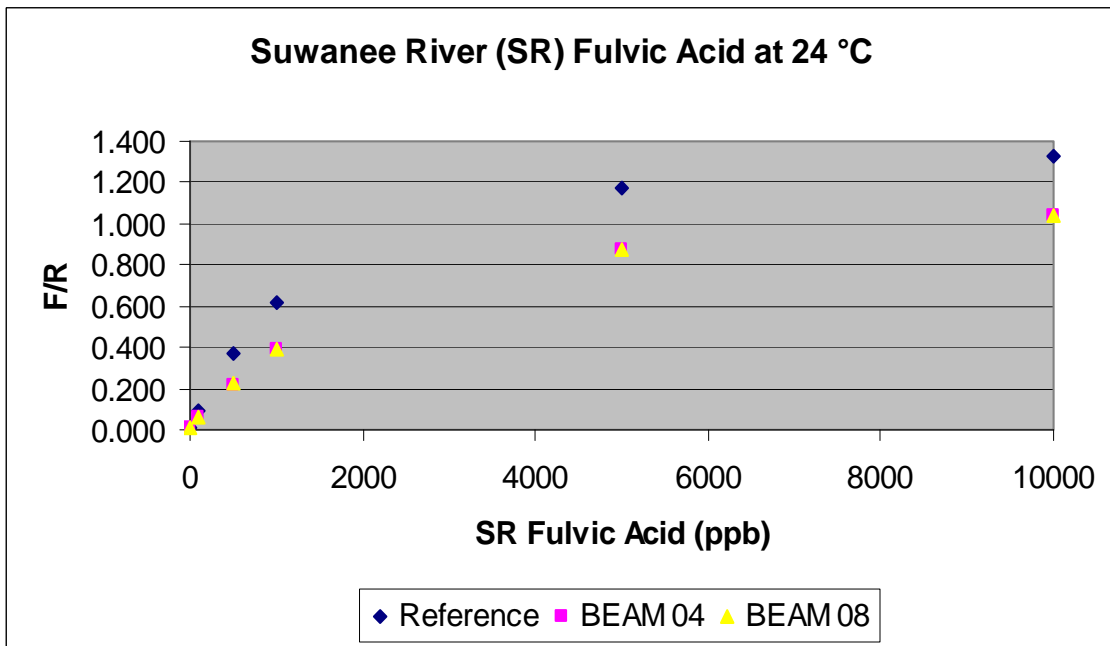
### 6.1 Accuracy

Accuracy was determined by comparing the BEAM 100 CDOM measurements (calculated as the intensity at 460 nm [F] divided by the intensity at 430 nm [R] or F/R) and the reference method F/R results generated by the Varian Cary Eclipse spectrometer for all PT and environmental sample analyses performed at 24°C. As shown in Figures 6-1, 6-2, and 6-3, the BEAM F/R measurements tracked the reference method F/R measurements, but were offset (i.e., the reference measurements ranged from approximately 8% to 26% higher than the BEAM measurements for quinine sulfate and from approximately 20% to 40% higher than the BEAM measurements for fulvic acid and environmental samples). Additionally the F/R measurements of both the BEAM and reference method plateau at higher concentrations, possibly due to internal quenching. The F/R ratios of the BEAM instruments and reference method instrument were not expected to be exactly the same because of differences in type and efficiency of gratings, detectors, the light source, and other conditions that vary from instrument to instrument. However, the instrumental differences can be partially compensated for by correlating the BEAM and reference method results based on the relationship between standards analyzed on each instrument. For ETV testing, quinine sulfate standards were used to generate a correlation between the BEAMs and the reference method. This relationship is shown in Figure 6-4, where the F/R measurements of both BEAM units are plotted against the reference method F/R measurements for quinine sulfate. Using the polynomial correlation between the BEAM F/R values and the reference method F/R values shown in Figure 6-4 [BEAM equivalent F/R = 0.1159\* (reference method F/R)<sup>2</sup> + 0.7031\* (reference method F/R)], the reference method F/R values for all of the test samples were converted to BEAM equivalent F/R values to assess the accuracy of the BEAM measurements in comparison to reference method measurements. Table 6-1 shows the PD between the BEAM equivalent reference method F/R values and the BEAM F/R values for each BEAM unit tested. The PD was <20% for all test samples except the unspiked solutions that were processed with the quinine sulfate and fulvic acid PT samples. The PD values of unspiked solutions are not representative because their very low F/R measurements result in small differences in F/R creating large PD values. In general, the results in Table 6-1 show that the lower the CDOM value, the greater the variability in the result. There was good agreement, however, between results for the same sample generated using two different BEAM units. Inter-unit reproducibility is discussed further in Section 6.5.

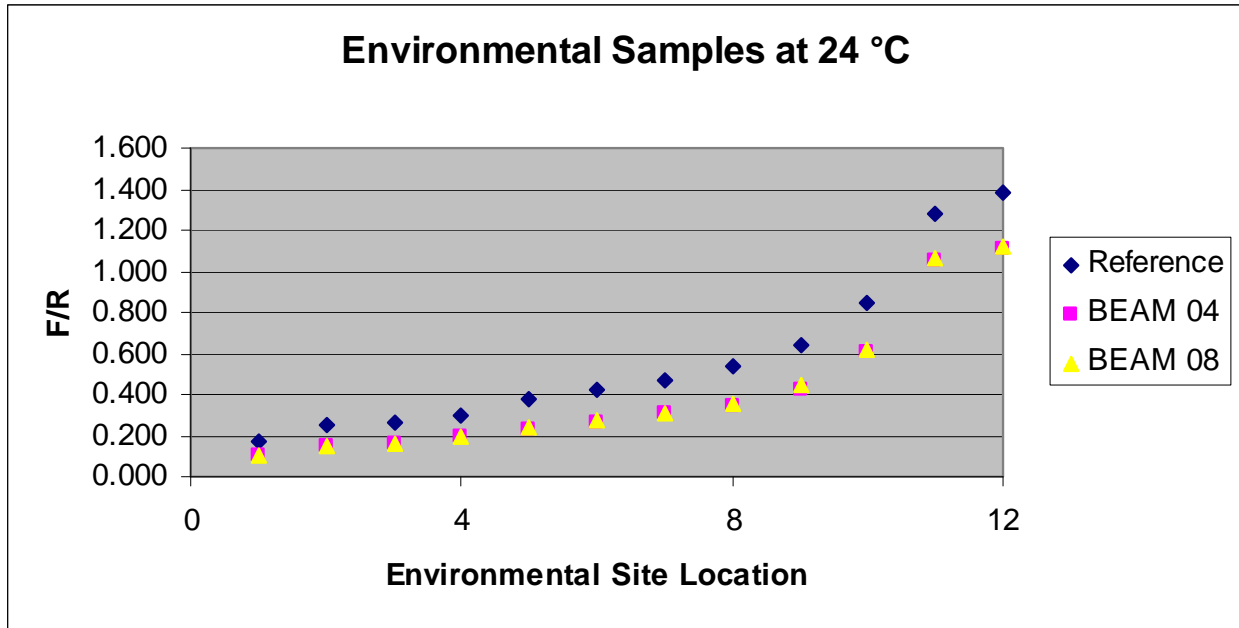




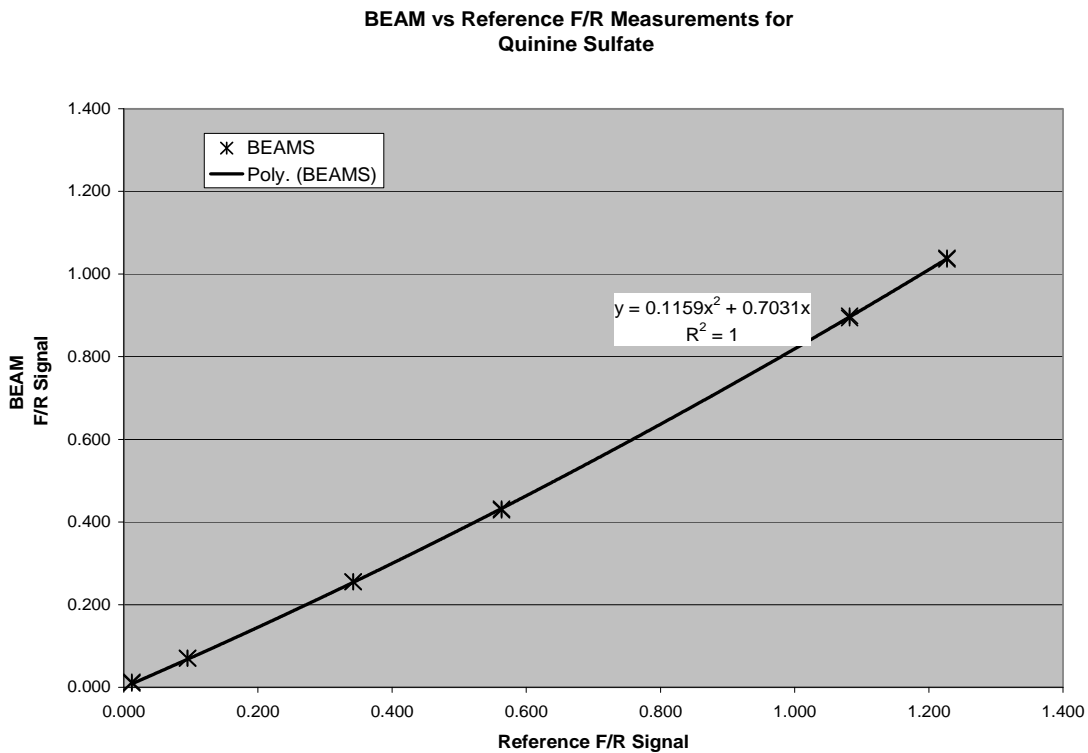
**Figure 6-1. Comparison of BEAM and Reference Method F/R Values for Quinine Sulfate (prior to correlating the BEAM and reference method results using quinine sulfate)**



**Figure 6-2. Comparison of BEAM and Reference Method F/R Values for SR Fulvic Acid (prior to correlating the BEAM and reference method results using quinine sulfate)**



**Figure 6-3. Comparison of BEAM and Reference Method F/R Values for Environmental Samples (prior to correlating the BEAM and reference method results using quinine sulfate)**



**Figure 6-4. Polynomial Correlation Between BEAM F/R and Reference Method F/R Measurements With Quinine Sulfate**

**Table 6-1. Percent Difference Between the BEAM F/R Values and the BEAM Equivalent Reference Method F/R Values**

	<b>PD BEAM 04 (%)</b>	<b>PD BEAM 08 (%)</b>
<b>Quinine Sulfate (ppb)</b>		
0	53.8	18.3
1	4.7	3.2
5	0.8	0.4
10	0.8	0.1
50	0.3	0.3
100	0.1	0.2
<b>SR Fulvic Acid (ppb)</b>		
0	41.9	9.2
100	11.1	11.1
500	19.9	18.8
1000	18.7	18.3
5000	11.8	11.3
10000	8.6	8.3
<b>Environmental Samples (listed in order of increasing F/R)</b>		
MOOS-1	11.6	14.0
East Coast, FL-2	17.4	17.9
Puget Sound, WA	16.1	15.0
NASS-5	11.3	12.2
San Diego Harbor, CA	16.8	15.3
Massachusetts Bay NF7	16.9	15.0
New York Bight, NY	15.0	13.6
Narragansett Bay, RI	16.9	13.2
Long Island Sound, NY	13.8	10.7
New York Harbor, NY	9.3	7.8
East Coast, FL-1	3.1	2.4
Boston Harbor, MA	7.9	6.2

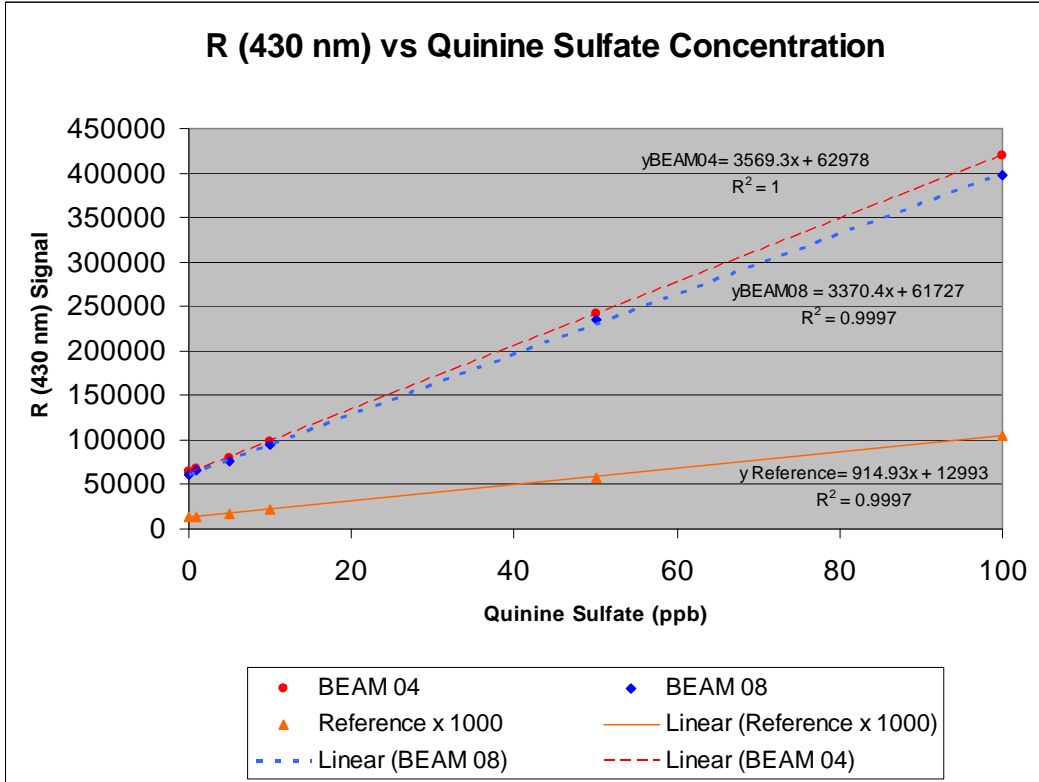
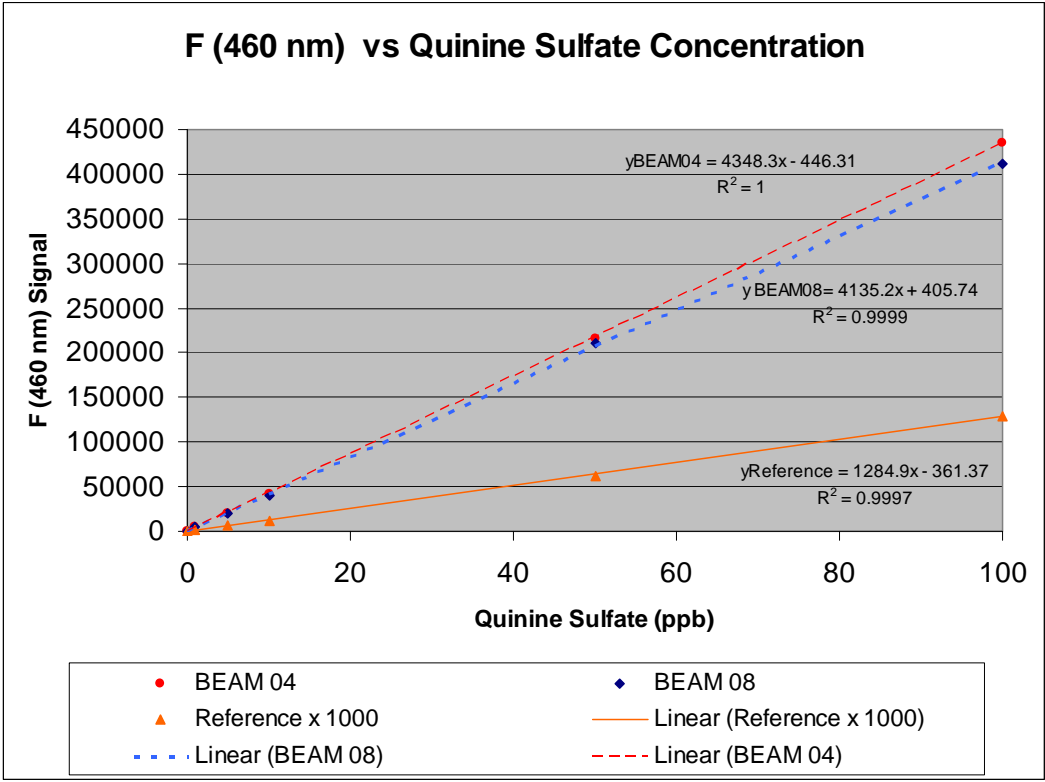
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While the BEAM and reference method F/R measurements track each other and are related to each other within a PD of 20% after correlating the BEAM and reference method measurements using quinine sulfate standards, this offset between BEAM and reference method F/R values may indicate that any action or cut-off F/R limits for BWE screening with the BEAM should be based on historical values for open-ocean or coastal water that have been generated on a BEAM and not on any other instrument unless the other instrument's data have been correlated to BEAM F/R values. This is discussed further in the matrix effects Section 6.7. Additionally, while the test/QA plan<sup>(4)</sup> defined using quinine sulfate to correlate the BEAM and reference method data due to its use as a calibration standard, it should be noted that quinine sulfate is not the only standard that could be used to relate the two different instruments. For example, a correlation could also have been made using the fulvic acid standards, or other standards could have been selected that might be more similar in composition to environmental samples. Use of different standards for correlation would result in different PD values for accuracy between the BEAM and reference methods for the various types of samples tested. For example, use of fulvic acid as the comparison standard would have likely improved PD for fulvic acid and environmental samples, while resulted in large PD values for quinine sulfate. For the purposes of ETV testing, only one standard, quinine sulfate, was used to correlate the BEAM to a reference method; however, it should be understood that quinine sulfate is not the only standard that could be used to correlate BEAM data to a reference method, nor is it necessarily the standard which most closely represents actual ballast water samples.

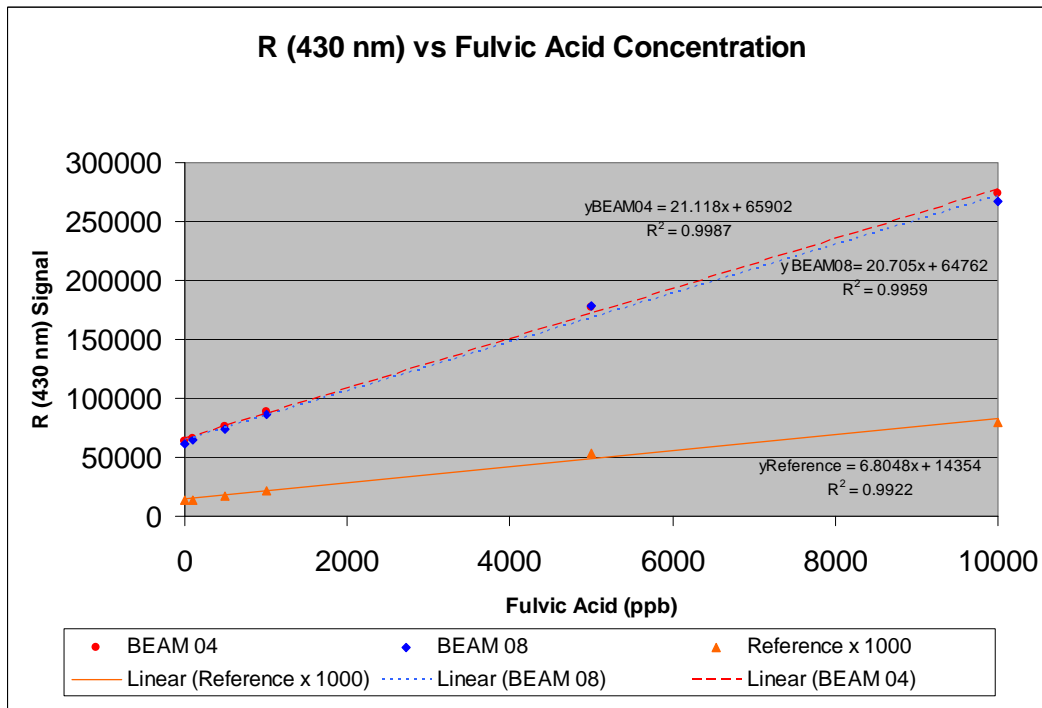
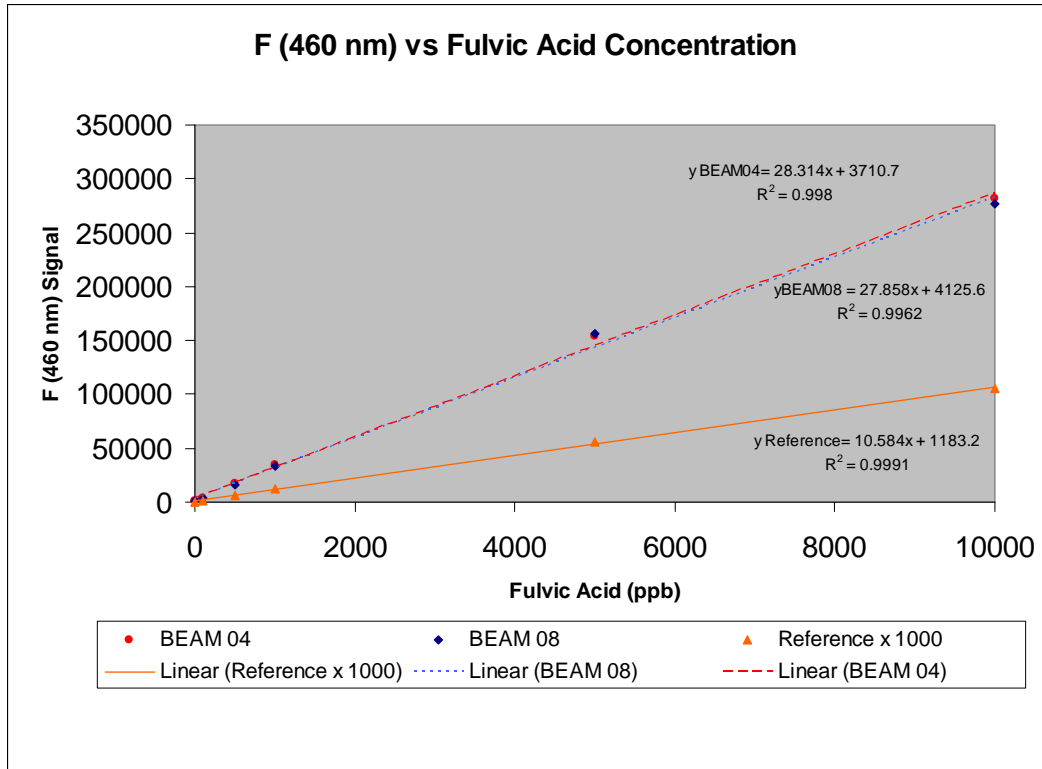
## 6.2 Linearity

Because the F/R ratio values plotted against concentration are nonlinear as evidenced in Figures 6-1, 6-2, and 6-3, linearity of the BEAM units as compared to the reference method was determined by plotting the individual F and R measurements while analyzing varying concentrations of analytes known to fluoresce (y-axis) against the analyte concentration (x-axis). Figures 6-5 and 6-6 show how the linearity of each BEAM unit F or R value compares with the linearity of the reference method F or R value. Because the signal output by the BEAM units and the reference method are of different intensity, the reference method values were multiplied by a factor of 1,000 to get the BEAM and reference F and R signals on the same scale. As demonstrated in Figures 6-5 and 6-6, both the BEAM and reference method F and R signals were linear across the concentration levels of quinine sulfate and fulvic acid analyzed, with  $R^2$  values greater than 0.99. The slopes of the BEAM and reference method signals are different, however. A difference in slope might be expected because of differences in the BEAM units and reference instrument (e.g., differences in type and efficiency of gratings, detectors, the light source, etc.).

Given the non-linearity of the F/R measurement with both the BEAM and reference method instruments (shown in Figures 6-1, 6-2, and 6-3) and that the BEAM reporting unit is the F/R ratio, it should be noted that it will be more difficult to distinguish between higher concentration CDOM samples that have higher F/R values. Any ballast water screening action limits would need to avoid the area in which the F/R ratios plateau.



**Figure 6-5. F (Upper Plot) and R (Lower Plot) Signals for the BEAM and Reference Method Plotted Against Quinine Sulfate Solution Concentration**



**Figure 6-6. F (Upper Plot) and R (Lower Plot) Signals for the BEAM and Reference Method Plotted Against Fulvic Acid Solution Concentration**

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### 6.3 Precision

The precision among triplicate measurements evaluated as RSD was comparable between the BEAM unit measurements and the reference method measurements at 24°C. Table 6-2 shows the RSD for each of the triplicate measurements made during verification testing. At 24°C, the RSDs of both the BEAM and reference measurements were less than 10%, with the exception of the unspiked fulvic acid solution analyzed with the reference method. Because the raw values of unspiked solutions were so low, small differences caused large RSDs. At the temperature extremes (4°C and 34°C) where only BEAM measurements and not reference method measurements were made, RSDs were less than 10% for all but the unspiked quinine sulfate solution (BEAM 03) and the 1-ppb quinine sulfate solution (BEAM 04). Again, the lower concentration-solution RSDs were affected by small differences in low-level measurements. The implication for BWE screening is that BEAM measurements for lower concentration CDOM samples, such as open-ocean samples, will be less precise than those for higher-concentration CDOM samples. However, out of 96 BEAM measurements evaluated for precision, only three had BEAM RSD values greater than 10%.

### 6.4 Method Detection Limit

MDLs were evaluated using both quinine sulfate and fulvic acid solutions by measuring seven replicates of each solution at concentrations five times the detection limit concentration specified by Dakota Technologies, Inc. Quinine sulfate MDLs were evaluated using a 1-ppb solution and fulvic acid MDLs were evaluated using a 100-ppb solution. MDL results for the BEAMs and reference method are listed in Table 6-3. Note that the reference method results were not adjusted to BEAM equivalent results using quinine sulfate for the MDL calculations. The calculated MDL F/R values following this 40 CFR 136 Appendix B<sup>(7)</sup> approach for both the BEAMs and the reference method are lower than the unspiked blanks analyzed with quinine sulfate and fulvic acid solutions, which had F/R measurements of approximately 0.01. It is possible that the MDL calculated in this way does not represent a practical detection limit, in part, because the water used to make up the standard solutions, as purified as it is, fluoresces above the MDL of the instruments. For the BEAMs, a practical MDL lies between the F/R values generated by the lowest concentration standards analyzed (F/R = 0.07 for 1 ppb quinine sulfate and F/R = 0.06 for fulvic acid) and the F/R of the unspiked blank samples (approximately 0.01) and is similar to the reference method (F/R = 0.09 for 1 ppb quinine sulfate, F/R = 0.1 for fulvic acid, and F/R = 0.01 for unspiked blank solutions).

### 6.5 Inter-unit Reproducibility

The RPDs between measurements of aliquots of the same test solution using two different BEAM units are shown in Table 6-4. Most measurements were within 10% RPD. However, the lower-concentration solutions that resulted in low CDOM F/R measurements were affected by small changes causing large RPDs. While RPDs were generally less than 10%, there was a noticeable increase in RPD for measurements at 4°C and 34°C compared with those at 24°C. Excluding the unspiked solutions, the RPDs between the two BEAM measurements of quinine sulfate and fulvic acid averaged 6.9% at 4°C and 6.6% at 34°C, compared to 0.6% at 24°C.

**Table 6-2. Relative Standard Deviation of Triplicate Measurements with BEAMs and Reference Method**

Sample Description		RSD (%)						
		24°C			4°C		34°C	
PT Samples		Reference Method	BEAM 04	BEAM 08	BEAM 04	BEAM 03	BEAM 04	BEAM 03
Quinine sulfate prepared in Burdick and Jackson HPLC grade water per ASTM E579-04 <sup>(5)</sup>	Unspiked	3.4	0.0	5.6	6.7	10.2	6.2	22.9
	1 ppb quinine sulfate	0.7	0.8	0.8	22.9	6.1	5.1	2.4
	5 ppb quinine sulfate	1.3	0.6	0.6	1.6	9.1	2.8	0.9
	10 ppb quinine sulfate	0.3	0.4	0.7	1.5	2.3	1.1	0.5
	50 ppb quinine sulfate	0.4	0.3	0.4	0.3	0.3	0.9	0.6
	100 ppb quinine sulfate	0.2	0.2	0.3	0.4	0.5	1.1	1.1
Fulvic acid prepared in Burdick and Jackson HPLC grade water	Unspiked	26.0	0.0	5.1	0.0	6.7	6.9	6.3
	100 ppb SR fulvic acid	2.6	0.0	2.0	3.5	6.0	1.3	0.9
	500 ppb SR fulvic acid	1.2	0.5	1.2	1.2	3.1	2.3	0.5
	1,000 ppb SR fulvic acid	1.6	0.9	0.6	1.2	1.6	0.8	2.4
	5,000 ppb SR fulvic acid	0.2	0.5	0.1	2.2	2.9	1.2	0.4
	10,000 ppb SR fulvic acid	0.1	0.7	0.3	0.9	1.3	2.5	1.9
Environmental Samples								
Location 1-open ocean	NASS-5	3.3	0.9	1.6				
Location 2-open ocean	MOOS-1	0.6	0.0	1.1				
Location 3-coastal	Long Island Sound, NY	1.0	0.4	0.5				
Location 4-coastal	New York Harbor, NY	0.5	0.4	0.1				
Location 5-coastal	New York Bight, NY	1.3	1.1	0.9				
Location 6-coastal	East Coast, FL-1	0.1	0.7	0.5				
Location 7-coastal	East Coast, FL-2	0.9	0.4	0.4				
Location 8-coastal	San Diego Harbor, CA	0.8	0.9	1.3				
Location 9-coastal	Narragansett Bay, RI	0.8	1.1	0.4				
Location 10-coastal	Puget Sound, WA	0.1	1.4	0.4				
Location 11-coastal	Massachusetts Bay NF7	1.1	1.6	0.6				
Location 12-coastal	Boston Harbor, MA	0.3	0.7	0.1				

Yellow highlight indicates RSD > 10%.



**Table 6-3. Method Detection Limits**

Sample Description		CDOM F/R Value		
		24°C		
PT Samples		Reference Method	BEAM 04	BEAM 08
1 ppb Quinine sulfate prepared in Burdick and Jackson HPLC grade water per ASTM E579-04 <sup>(5)</sup>	REPLICATE-1	0.0895	0.071	0.071
	REPLICATE-2	0.0955	0.072	0.072
	REPLICATE-3	0.0936	0.073	0.072
	REPLICATE-4	0.0930	0.072	0.071
	REPLICATE-5	0.0952	0.072	0.072
	REPLICATE-6	0.0939	0.071	0.071
	REPLICATE-7	0.0962	0.072	0.070
	SD	0.0022	0.00069	0.00076
	MDL	0.0070	0.0022	0.0024
	Unspiked blank	0.012	0.013	0.010
100 ppb Fulvic acid prepared in Burdick and Jackson HPLC grade water	REPLICATE-1	0.1001	0.062	0.064
	REPLICATE-2	0.1002	0.062	0.062
	REPLICATE-3	0.1001	0.062	0.062
	REPLICATE-4	0.0999	0.062	0.061
	REPLICATE-5	0.1003	0.061	0.060
	REPLICATE-6	0.0981	0.062	0.061
	REPLICATE-7	0.0979	0.061	0.058
	SD	0.0011	0.00049	0.0019
	MDL	0.0033	0.0015	0.0059
	Unspiked blank	0.013	0.013	0.010

Because one BEAM unit (BEAM 08) was exchanged between the 24°C testing and the temperature extreme testing (BEAM 03), it is not clear whether the differences in RPD values result from the temperature extremes or other differences between BEAM 08 and BEAM 03. However, the inter-unit reproducibility was generally within 10% overall.

## 6.6 Temperature Effects

The BEAM units and the test solutions were equilibrated at 4°C and 34°C for a minimum of 30 minutes prior to taking any measurements. Once equilibrated at the appropriate temperature, the BEAM units were calibrated with the 10-ppb quinine sulfate calibration solution. The calibration procedure set the F/R value for the 10-ppb quinine sulfate calibration solution at a value of 0.43. The upper plot in Figure 6-7 shows that, at 4°C, the BEAM quinine sulfate measurements agreed quite well with the 24°C measurements. At 34°C, however, the BEAM quinine sulfate measurements begin to diverge from the 24°C measurements, especially at F/R values greater than 0.5. This is also shown in Table 6-5, which lists the PD values between the temperature extreme measurements and the 24°C measurements. When measurements at 4°C are compared with those at 24°C for quinine sulfate, all PD values are less than 10%, with the exception of the unspiked solution. However, when measurements at 34°C are compared with those at 24°C, the PD values are less than 10% only for the 5-ppb and 10-ppb quinine sulfate solutions, which had F/R values near the 0.43 F/R calibration level. As the F/R values move away from the calibrated 0.43 level, the PD between the 34°C and 24°C measurements

**Table 6-4. BEAM Inter-unit Reproducibility**

		RPD (%)		
		24°C	4°C	34°C
<b>PT Samples</b>		<b>BEAM 04 vs. BEAM 08</b>	<b>BEAM 04 vs. BEAM 03</b>	<b>BEAM 04 vs. BEAM 03</b>
Quinine sulfate prepared in Burdick and Jackson HPLC grade water per ASTM E579-04 <sup>(5)</sup>	unspiked	26.1	14.0	92.3
	1 ppb quinine sulfate	1.4	12.4	17.2
	5 ppb quinine sulfate	0.4	7.5	2.2
	10 ppb quinine sulfate	0.7	10.4	5.8
	50 ppb quinine sulfate	0.6	5.5	0.8
	100 ppb quinine sulfate	0.3	7.6	0.8
Fulvic acid prepared in Burdick and Jackson HPLC grade water.	unspiked	26.1	18.2	57.8
	100 ppb SR fulvic acid	0.0	3.4	20.7
	500 ppb SR fulvic acid	1.3	6.2	9.8
	1000 ppb SR fulvic acid	0.5	4.3	3.2
	5000 ppb SR fulvic acid	0.6	7.8	0.0
	10000 ppb SR fulvic acid	0.3	4.0	5.9
<b>Environmental Samples</b>				
Location 1-open ocean	NASS-5	1.0		
Location 2-open ocean	MOOS-1	2.8		
Location 3-coastal	Long Island Sound, NY	3.5		
Location 4-coastal	NY Harbor, NY	1.6		
Location 5-coastal	NY Bight, NY	1.6		
Location 6-coastal	East Coast FL-1	0.8		
Location 7-coastal	East Coast FL-2	0.7		
Location 8-coastal	San Diego Harbor, CA	1.7		
Location 9-coastal	Narragansett Bay, RI	4.3		
Location 10-coastal	Puget Sound, WA	1.2		
Location 11-coastal	Massachusetts Bay NF7	2.2		
Location 12-coastal	Boston Harbor, MA	1.8		

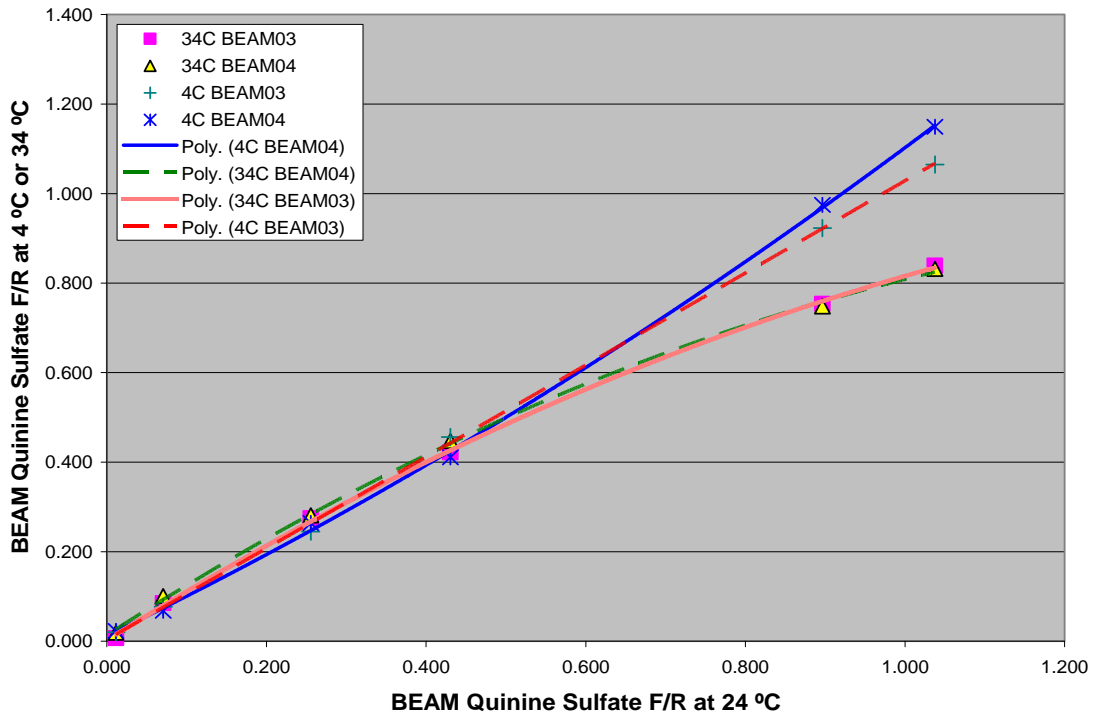
Yellow highlight indicates RPD > 10%.

increases. This divergence away from the 0.43 calibration level illustrates that a sample's fluorescence differs with temperature. This is a typical fluorescence phenomenon, with lower temperatures increasing fluorescence and higher temperatures decreasing fluorescence, and should not be interpreted as a function of the BEAM units.

Similar comparisons for fulvic acid are shown in the lower plot in Figure 6-7 and the PD data listed in Table 6-5. As for quinine sulfate, fulvic acid measurements at 34°C agree well near the calibrated 0.43 F/R level, but the PD increases as the F/R values move away from the calibrated 0.43 level.

At 4°C, considerable PD (26% and 98%) exists between the fulvic acid F/R values and the 24°C measurements across all solutions tested.

### BEAM Quinine Sulfate Measurements at 4 °C and 34 °C vs 24 °C



### BEAM Fulvic Acid Measurements at 4 °C and 34 °C vs 24 °C

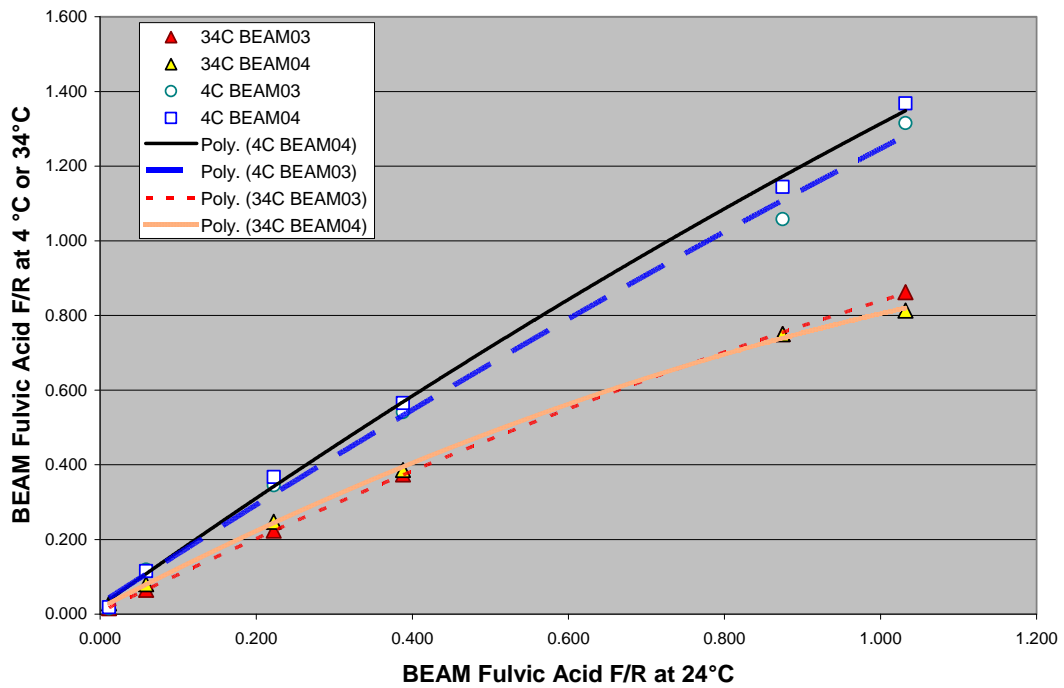


Figure 6-7. Comparison of BEAM F/R Values for Quinine Sulfate (Upper Plot) and Fulvic Acid (Lower Plot) at 4°C and 34°C to Those at 24°C

**Table 6-5. Percent Difference of 4°C and 34°C BEAM Measurements from 24°C BEAM Measurements**

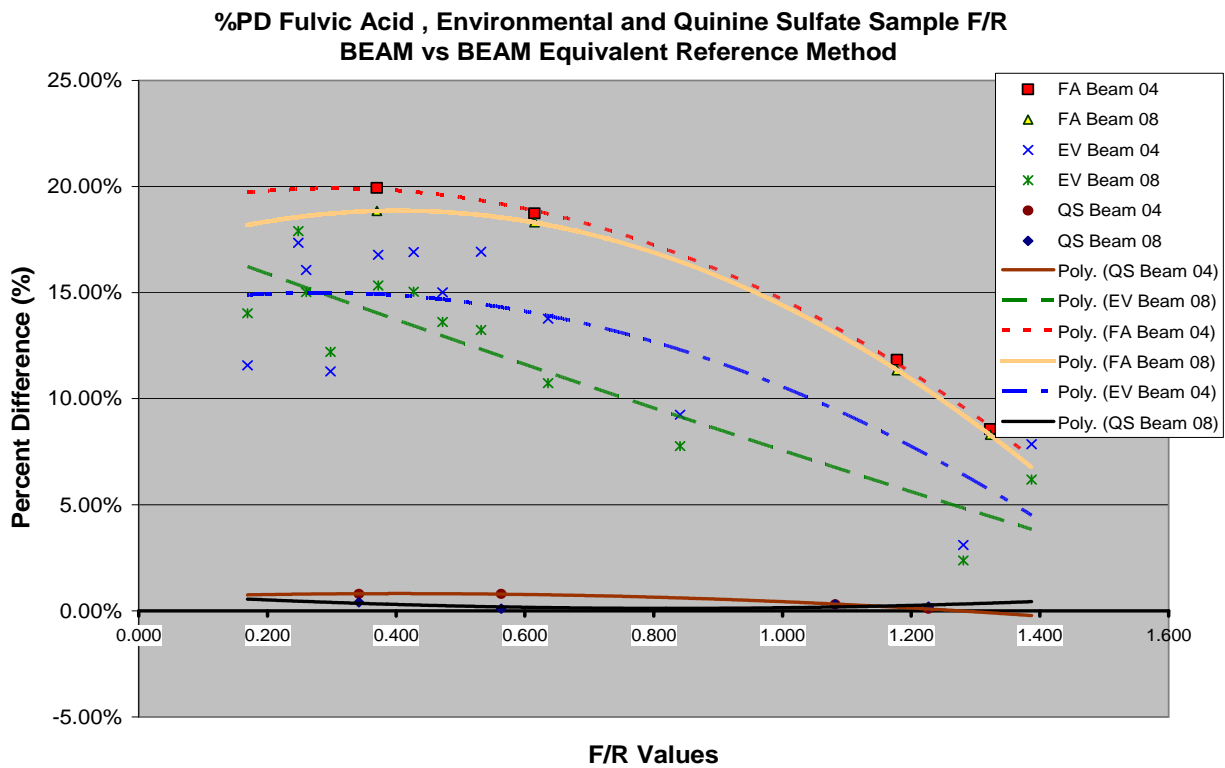
Sample Description		Percent Difference (%)	
		Avg. BEAM 03/04 at 4°C vs. Avg. BEAM 08/04 at 24°C	Avg. BEAM 03/04 at 34°C vs. Avg. BEAM 08/04 at 24°C
<b>PT Samples</b>			
Quinine sulfate prepared in Burdick and Jackson HPLC grade water per ASTM E579-04 <sup>(5)</sup>	Unspiked	87.0	13.0
	1 ppb quinine sulfate	2.8	31.9
	5 ppb quinine sulfate	0.4	8.8
	10 ppb quinine sulfate	0.7	0.9
	50 ppb quinine sulfate	5.9	16.2
	100 ppb quinine sulfate	6.7	19.5
Fulvic acid prepared in Burdick and Jackson HPLC grade water	unspiked	43.5	95.7
	100 ppb SR fulvic acid	98.3	22.9
	500 ppb SR fulvic acid	60.0	5.8
	1,000 ppb SR fulvic acid	42.5	2.1
	5,000 ppb SR fulvic acid	25.9	14.2
	10,000 ppb SR fulvic acid	30.0	18.8

These results illustrate the importance of calibrating the BEAM units at the testing temperature and ensuring that the calibration solution has an F/R close to any action level developed for BWE screening to maximize the accuracy of measurements near action-level concentrations. The results also underscore the fact that temperature can cause deviations in F/R values in the PT sample solutions, particularly when the solution F/R values differ from the 0.43 calibration level. Additionally, the differences between the quinine sulfate and fulvic acid F/R responses at the various temperatures suggest that temperature effects may vary depending on the sample composition.

## 6.7 Matrix Effects

The PD data in Table 6-1 (excluding the data for the unspiked blanks), which is also displayed graphically in Figure 6-8, show that fluorescence observed by the BEAM and reference method instruments is affected by the matrix. As noted in Section 6.1 above, the reference method results were converted to BEAM equivalent results based on the correlation of the BEAM and reference method when analyzing quinine sulfate. Using the reference method results adjusted based on the quinine sulfate correlation, the environmental samples and the fulvic acid samples yield a different relationship to the reference method measurements (PDs ranging from approximately 2 to 20%) than the quinine sulfate samples (all PDs less than 5%). Differing results based on matrix are not unexpected based on instrumental differences between the BEAM and the reference method (i.e., use of gratings in the reference method instrument versus using filters in the BEAM) and how different compounds fluoresce. The amount of fluorescence depends upon the amount of absorption occurring with all of the species at the excitation wavelength. The more spectroscopically complex the matrix, the greater the variability is likely to be. For example, compounds present in the fulvic acid and environmental samples may obscure the excitation energy, causing the fluorescing compounds to fluoresce less than with a simple standard such as quinine sulfate. The composition of different environmental samples can also result in different amounts of fluorescence quenching. Changes in quenching will change

both shape and intensity of fluorescence signals. Seawater is a very complex mixture of compounds, and so matrix effects could be expected.



**Figure 6-8. Matrix Effects Based on Percent Difference of BEAM Results Compared with BEAM Equivalent (Based on Quinine Sulfate Correlation) Reference Method Results**

When using quinine sulfate to correlate the BEAM to the reference method, the differences in the way the two instruments respond to different matrices (and not compounding with temperature effects, etc.) result in the environmental sample BEAM F/R values varying approximately 15% from the Varian Cary BEAM equivalent (quinine sulfate adjusted) reference method F/R values for samples with F/R values <0.6. As discussed previously, the F/R ratios of the BEAM and reference method instruments were not expected to be the same because of differences in type and efficiency of gratings, detectors, the light source, and other conditions that vary from instrument to instrument. This would be the case between any hand-held fluorimeter compared to any laboratory bench-top spectrometer and is not unique to the BEAM. The implication of this for BWE screening is that, among instruments, target values likely will be different for each instrument design. This implies a need to set BWE action limits on an instrument specific basis. Likewise, any comparison of screening instrument results to those generated using a laboratory based reference method will be more accurate by correlating the screening instrument to the reference method based on the relationship between the same standards analyzed on each instrument, as was conducted for this verification test. While quinine sulfate was used for this verification test, it is beyond the scope of this test to determine what standard serves as the best for correlating ballast water screening results between different instruments for any kind of regulatory purpose.

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## 6.8 Data Completeness

Data completeness for this verification test was 100%. All data measurements expected to be taken were completed and were usable.

## 6.9 Operational Factors

The BEAM units were easy to operate. The data display was easy to read, and the data were easy to download to a PC. Testing staff received a 4-hour training session from Dakota Technologies, Inc., which was more than sufficient to familiarize the staff with BEAM operation and data downloading procedures. The BEAM units contained an instruction manual with clearly written information and illustrations. While the instructions for each specific procedure such as blank calibrations, quinine sulfate calibrations, and calibration checks were easy to follow, more information on required frequency of each procedure and QC pass/fail criteria for the procedure would be useful to ensure that the operator knows whether the BEAM units are functioning properly. Such limits were agreed upon with Dakota Technologies, Inc. for use during verification testing (e.g., acceptable CDOM measurement ranges for the calibration checks, maximum readings for negative control samples, etc.) and were useful for ensuring that instrumentation was clean and operating properly. Contents of the BEAM kit were listed in the instruction manual; however, it would be useful for the actual vial containing cleaning solution to be labeled. From a safety perspective, it would also be useful to identify for the user, either on the vial or in the instruction manual, any hazard associated with the cleaning solution or special precautions necessary in case of spillage or user exposure. Instructions include information for storing the BEAM cell with clean water to prevent spotting, but no information for storing the cleaning solution. Given the potential for the BEAM units to be used at temperature extremes during practical application, guidance as to any precautions for storage under such conditions may be useful. Storage conditions for the quinine sulfate calibration solution, which is not included in the BEAM kit but needed for operation, are discussed in the ASTM method referenced in the instruction manual, but are not discussed in the instruction manual itself.

All items included in the BEAM kit were easy to open. The Luer-lock syringe provided with the BEAM kit required considerable hand strength for processing large quantities of samples. A user may want to investigate other types of syringes if planning to process more than two or three samples in a short time period. Reagents were easy to prepare; however, since the 10-ppb quinine sulfate calibration solution was made daily following the ASTM guidance, preparation of calibration solution took a fair amount of time each analysis day during verification testing. With the exception of the 10-ppb quinine sulfate calibration solution and distilled water for rinsing, all reagents were supplied with the kit.

The BEAM kit provided a container for rinse water, but the BEAM carrying case did not provide for the calibration solution. Assuming that the calibration solution must be carried along with the BEAM for use in the field, a container or holding spot for this solution in the carrying case would be useful. The BEAM kit included all necessary equipment, with the exception of pipettes, flasks, balances, and containers used to prepare and store the quinine sulfate calibration solution and to store waste. The BEAM unit's exterior was easily wiped clean. Other than keeping the cell clean, no routine maintenance was required. Approximately 12 milliliters (mL) of sample waste and 20 mL of water rinse waste were generated with every sample measurement.

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The number of samples that can be processed continuously with a BEAM, assuming unlimited access to distilled water for rinsing the BEAM cells, is limited by the life of the batteries in the BEAM and by the BEAM's internal memory size. During verification testing, the six AA batteries were replaced once after processing approximately 100 samples. A spare set of batteries in the BEAM kit would be useful. Spare batteries were supplied in the BEAM kits provided for verification testing, but are not listed in the instruction manual as standard items with the kit. The BEAM's internal memory will hold 256 measurements before it overwrites previous readings. This is not an issue if data are also recorded manually or downloaded to a PC. If the operator must rely only on the distilled water in the provided rinse bottle, approximately 15 samples could be processed before more water would be needed. This estimate assumes that the cell and cap are rinsed with water twice between each analysis. Overall sample throughput was between 20 and 25 samples per hour (approximately three samples per minute) when manually recording the CDOM F/R value and properly rinsing the cell between each sample reading.

During verification testing, some technical difficulties were encountered with the data displays and system interlocks. For example, after tightening down the cell cap, an interlock countdown occurs before a measurement reading can be taken. This interlock prevents light-leaks that could impact measurements. However, in some instances, this interlock countdown would repeat multiple times before a measurement reading would be taken, even though the cap was tightly secured. Display errors observed during verification testing included the 460-nm column header sometimes not displaying properly and calibration values sometimes appearing in the display instead of sample results. The frequency of technical difficulties increased when operating at the temperature extremes. At 34°C, one of the BEAM units (BEAM 08) had unusual calibration results with the 10-ppb quinine sulfate solution and subsequently generated system errors every time a sample analysis was attempted. When cooled to 24°C, the calibration and sample readings were successful, but failed again when exposed to 34°C a second time. Similar problems with this unit occurred with 4°C testing. This particular unit was replaced before the 34°C and 4°C testing. Dakota Technologies, Inc. provided phone support for troubleshooting and replacement equipment when difficulties were encountered during verification testing. The replacement BEAM unit (BEAM 03) did not have problems at the temperature extremes; however, this unit did have difficulties with the interlock issues described above. The number of BEAM units tested was too small to determine whether the problems with BEAM 08, potentially induced by temperature extremes, is a weakness in the instrumentation or a random, chance instrument failure. It also should be noted that the BEAMs power down after 7 minutes of idle time and then require a 90-count warm-up period before restarting, which can add to analysis time.

## Chapter 7 Performance Summary

**Table 7-1. BEAM 100 Summary Table**

Performance Factor	Sample Information	Result
Accuracy	Five concentrations of quinine sulfate prepared in Burdick and Jackson HPLC grade water per ASTM E579-04 <sup>(5)</sup> plus one unspiked blank; five concentrations of SR fulvic acid plus one unspiked blank; and 12 environmental (natural water) samples. All testing was performed at approximately 24°C.	PD from reference method measurements (using a quinine sulfate <sup>A</sup> correlation between the BEAM and reference method results) was less than 20% for both quinine sulfate and fulvic acid samples, except for the unspiked, blank samples. PD was also less than 20% for environmental samples. PD values increased with lower CDOM measurements.
Linearity	Five concentrations of quinine sulfate prepared in Burdick and Jackson HPLC grade water per ASTM E579-04 <sup>(5)</sup> plus one unspiked blank; five concentrations of SR fulvic acid plus one unspiked blank. All testing was performed at approximately 24°C.	Individual signals at 460 nm and 430 nm were linear across the concentrations tested and had R <sup>2</sup> values >0.99 for both quinine sulfate and fulvic acid test solutions.
Precision	Five concentrations of quinine sulfate prepared in Burdick and Jackson HPLC grade water per ASTM E579-04 <sup>(5)</sup> plus one unspiked blank; five concentrations of SR fulvic acid plus one unspiked blank; and 12 environmental (natural water) samples. Testing was performed at approximately 24°C, 4°C, and 34°C.	RSD of triplicate measurements of each test sample was <10% except for low CDOM concentration samples such as the unspiked blank samples for which the highest RSD was 22.9%.
MDL	Seven replicates of 1 ppb quinine sulfate and seven replicates of 100 ppb SR fulvic acid analyzed following 40 CFR 136 Appendix B <sup>(7)</sup> procedures. Concentrations were set at five times the vendor-specified detection limit for each compound. All testing was performed at approximately 24°C.	Calculated MDLs were lower than the CDOM values of the unspiked blank samples (<0.01) and, therefore, may not represent practical detection limits. The BEAMs proved capable of detecting CDOM values <0.06 to 0.07, which were the CDOM values of the lowest concentration quinine sulfate and fulvic acid standards analyzed.



## 7-1. BEAM 100 Summary Table (continued)

Inter-unit Reproducibility	All test samples. Testing was performed at approximately 24°C, 4°C, and 34°C.	RPD values between the average of triplicate measurements were mostly <10% at all testing temperatures. RPD increased as CDOM concentration decreased.
Temperature Effects	Five concentrations of quinine sulfate prepared in Burdick and Jackson HPLC grade water per ASTM E579-04 <sup>(5)</sup> plus one unspiked blank; five concentrations of SR fulvic acid plus one unspiked blank. Testing was performed at temperature extremes of approximately 4°C and 34°C and compared with results obtained at approximately 24°C (ambient conditions).	For the spiked samples, PD values ranged as follows: QS solutions: 0.4 to 6.7% for 4°C vs 24°C 0.9 to 31.9% for 34°C vs 24°C SRFA solutions: 25.9 to 98.3% for 4°C vs 24°C 2.1 to 22.9% for 34°C vs 24°C For the unspiked blanks, the PD values ranged from 13.0 to 95.7%. The results indicate that temperature changes can cause deviations in performance and illustrate the importance of calibrating the BEAM units at the testing temperature.
Matrix Effects	Five concentrations of quinine sulfate prepared in Burdick and Jackson HPLC grade water per ASTM E579-04 <sup>(5)</sup> plus one unspiked blank; five concentrations of SR fulvic acid plus one unspiked blank; and 12 environmental (natural water) samples. All testing was performed at approximately 24°C. The accuracy PD measurements comparing BEAM CDOM to reference method measurements (using a quinine sulfate correlation between the BEAM and reference method results) of the same solution were evaluated for differences between matrix type.	Distinct differences in correlation to reference method values were observed based on matrix type. Environmental samples and fulvic acid samples were between 2 and 20% PD from BEAM equivalent reference method measurements (using a quinine sulfate <sup>A</sup> correlation between the BEAM and reference method results), whereas quinine sulfate samples were all less than 5% PD.
Data Completeness	All test samples.	Data completeness was 100%. All intended analyses and measurements were performed and all measurements were valid and usable.
Operational Factors	The BEAM 100 units were portable, convenient, easy to use, and came with a clearly written instruction manual. Sample throughput was 20 to 25 samples per hour. Waste generated while processing each sample included approximately 12 mL of sample waste and 20 mL of water rinse waste. Factors limiting continuous operation of the BEAM include battery life (six AA batteries had to be replaced after ~100 measurements), BEAM internal memory size (data are overwritten after 256 measurements), access to distilled water (rinse bottle provided with BEAM holds enough distilled water for ~15 samples), and operator hand strength (each sample must be filtered through a 0.45-micron filter using a Luer-lock syringe). Technical difficulties with displays and system interlocks resulted in one BEAM unit being replaced by the vendor during testing. Technical difficulties increased when testing at approximately 4°C, and 34°C. Not enough BEAM units were evaluated to know whether these technical difficulties indicate more than a random instrument failure.	

<sup>A</sup> Quinine sulfate was selected to correlate the BEAM and reference instruments because of its use as a spectroscopic standard. Use of other standards with properties closer to the environmental samples may have improved PD values for the environmental samples; however, this was not verified as part of this test.

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## Chapter 8 References

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