

**PROCEDURES AND CRITERIA  
ANALYSIS OF OPTICAL BRIGHTENER  
ON COTTON SAMPLERS**

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## **PROCEDURES**

### **Introduction**

This document describes standard procedures and criteria currently in use at the Ozark Underground Laboratory as of the date shown on the title page. Some samples may be subjected to different procedures and criteria because of unique conditions; such non-standard procedures and criteria are identified in reports for those samples. Standard procedures and criteria change as knowledge and experience increases and as equipment is improved or up-graded. The Ozark Underground Laboratory maintains a summary of changes in standard procedures and criteria.

### **Optical Brightener Nomenclature**

Optical brighteners are fluorescent white dyes found in laundry soaps and detergents.

### **Description of the Samplers**

The samplers used by the Ozark Underground Laboratory are 6 by 6 inch pieces of cotton test fabrics that have not been optically brightened. These fabric squares are Testfabrics, Inc style 400U unbleached cotton print cloth.

### **Placement of Samplers**

These are cumulative samplers; they are placed in the water to be sampled for a period of up to about a week. Sampling is enhanced when a small volume of new water is allowed to continuously contact the sampler.

### **Collection and Replacement of Samplers**

Samplers are routinely collected and replaced from each of the sampling stations. The frequency of sampler collection and replacement is determined by the nature of the study. Collections at one-week intervals are common.

Where convenient, the collected samplers should be briefly rinsed in the water being sampled. The samplers are placed in plastic bags (Whirl-Pak bags are ideal). The bag is labeled on the outside with a permanent type felt marker pen. The notations include station name or number and the date and time of collection. Labels are not inserted inside the sample bags.

Collected samplers are kept in the dark to minimize algal growth prior to analysis work. We prefer that samples be placed on "blue ice" or ice upon collection and that they be shipped refrigerated with "blue ice" by overnight express. Our experience indicates that it is not essential for samplers to be maintained under refrigeration, yet maintaining them under refrigeration clearly minimizes some potential problems.

A chain of custody or sample tracking sheet must accompany each shipment of samplers. These sheets (which bear the title "SAMPLE COLLECTION DATA SHEET for OPTICAL BRIGHTENER ANALYSIS") are provided by the Ozark Underground Laboratory and summarize placement and collection data. These sheets can be augmented by a client's chain of custody forms or any other relevant documentation. Figure 1 is one of our blank sample collection data sheets.



## **Receipt of Samplers**

Samplers shipped to the Ozark Underground Laboratory are refrigerated upon receipt. Prior to cleaning and analysis, samplers are assigned a laboratory identification number. All samples are logged in upon receipt and are recorded in a bound journal.

It sometimes occurs that there are discrepancies between the chain-of-custody sheets and the actual samples received. When this occurs, a "Discrepancy Sheet" form is completed and included with the Certificate of Analysis. A copy of this form is enclosed as Figure 2. The purpose of the form is to help resolve discrepancies, even when they may be minor.

## **Cleaning of Samplers**

Samplers are cleaned by spraying them with jets of clean water. At the Laboratory we use unchlorinated water for the cleansing to minimize dye deterioration. Effective cleansing cannot generally be accomplished simply by washing in a conventional laboratory sink even if the sink is equipped with a spray unit.

Next, the samplers are scanned under an ultraviolet lamp to identify surface areas most likely to contain optical brighteners. Any areas identified as likely to contain optical brighteners are oriented in the spectrofluorometer so as to be included in the analysis.

## **Analysis on the Shimadzu RF-540 or RF5000U**

The Laboratory uses both a Shimadzu RF-540 and a Shimadzu RF-5000U Spectrofluorometer capable of synchronous scanning. The RF-540 is the primary instrument used; the RF-5000U is the back-up instrument.

Our instruments are operated and maintained in accordance with the manufacturer's recommendations. Delta Instrument Company, the dealer for Shimadzu Instruments, provided on-site installation of the instruments and training sessions on the instruments.

The cotton sampler is placed in the RF-540 or the RF-5000U. A programmable computer controls both instruments. Each instrument is capable of conducting substantial data analysis.

The samplers are placed in a solid sample holder behind a quartz glass slide for analysis. The samplers are oriented so that likely areas of fluorescence will be included in the analysis.

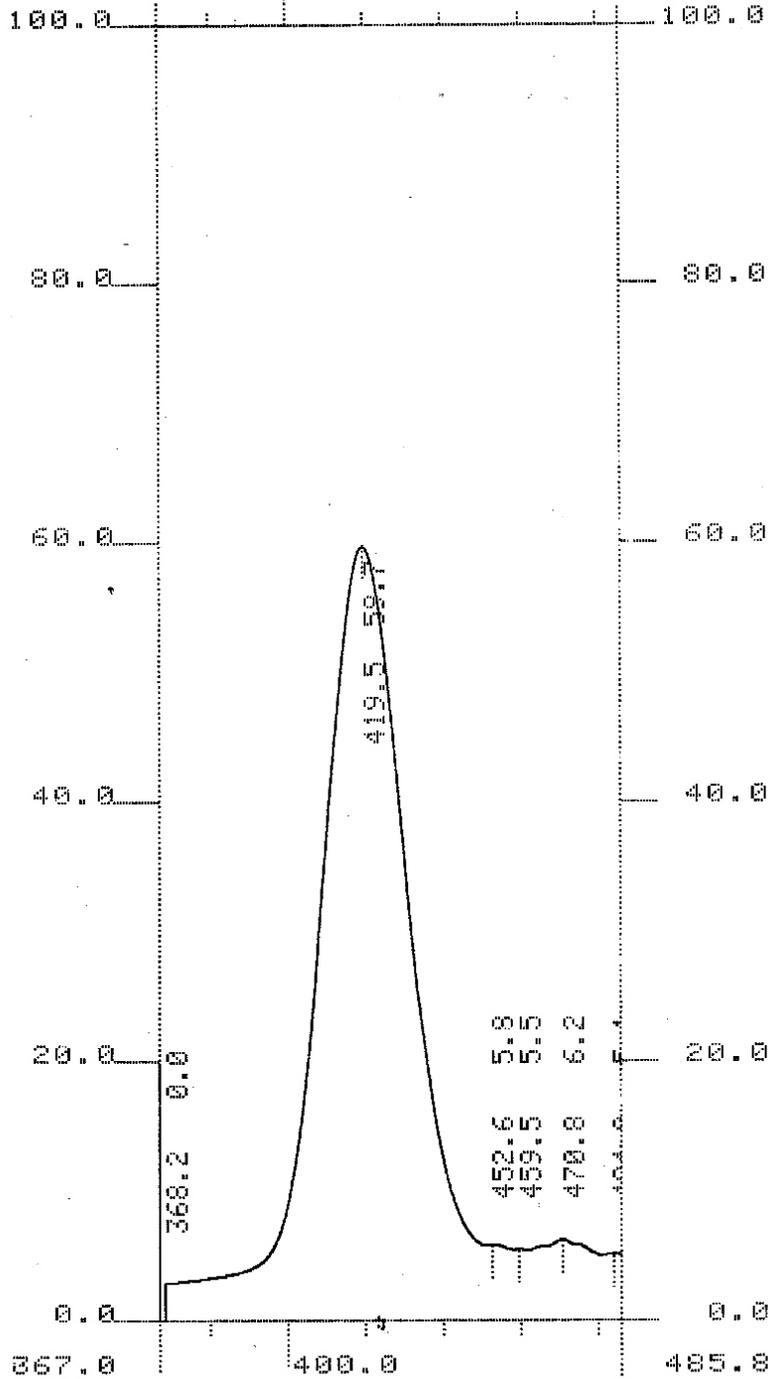
Our typical analysis of a cotton sampler where optical brightener may be present includes synchronous scanning of excitation and emission spectra with a 17 nm separation between excitation and emission. The excitation scan is from 350 to 470 nm; the emission scan is from 367 to 487 nm. The emission fluorescence from the scan is plotted on a graph. The typical scan speed is "very fast;" typical sensitivity is "high."

The excitation slit for cotton samplers is typically 5 nm, the emission slit is typically 2 nm on the RF-540 and 3 nm on the RF-5000U. This is because the RF-540 does not provide a 3 nm slit setting and the RF-5000U does not provide a 2 nm slit setting. The scales are typically set so as to keep the resulting chart to a size that can readily be photocopied and to provide good data resolution while not exceeding the upper limit of the resulting graph. The initial abscissa scale setting is X4 and the initial ordinate scale setting is X16.

The instrument produces a plot of the synchronous scan for each sample; the plot shows only emission fluorescence as shown in Figure 3. It is photocopied as a part of the final record. All samples analyzed are recorded in a bound journal.



Figure 3.



LIST OF PARAMETER

ABSCISSA SCALE	X4	ORDINATE SCALE	X4
367.0	VERY FAST	0.0	HIGH
368.2	5	0.0	2
419.5	5	59.7	2
452.6	5	5.0	2
459.5	5	5.5	2
470.0	5	6.2	2
487.0	5	0.0	2

EXCITATION WAVELENGTH	EMISSION WAVELENGTH
470.0	487.0
350.0	470.0
367.0	487.0

The original RF-540 plots are on pressure sensitive chart paper. During analysis, identification numbers and other notes are made on the original charts. All samples run on the RF-5000U are stored on disk and printed on normal typing paper with a laser printer; sample information is printed on the chart.

### **Quantitative and qualitative results**

We routinely report both quantitative and qualitative results. The peak wavelength and the height of the fluorescence peak are reported for each sample found to contain optical brightener. Additionally, a qualitative result is provided for each sample analyzed. Those qualitative results are defined as follows:

ND None detected: No fluorescence peak detected in the acceptable wavelength range of optical brighteners (415.0 to 422.0 nm).

WP Weakly Positive. A small fluorescence peak was detected in the acceptable wavelength range of optical brighteners (415.0 to 422.0 nm). The peak is small and of inadequate magnitude to obscure other non-optical brightener peaks in the range of 407.0 to 412.0 nm. The peak has a fluorescence height of 15 fluorescence units or less.

MP Moderately Positive. A fluorescence peak is detected in the acceptable wavelength range of optical brighteners (415.0 to 422.0 nm). The height of the peak is more than 15 but less than 50 fluorescence units. The peak is of adequate magnitude to obscure other non-optical brightener peaks in the range of 407.0 to 412.0 nm.

SP Strongly Positive. A fluorescence peak is present in the acceptable wavelength range of optical brighteners (415.0 to 422.0 nm). The height of the peak is 50 fluorescence units or more. The peak is of adequate magnitude to obscure other non-optical brightener peaks in the range of 407.0 to 412.0 nm.

### **Quality Control**

A random sample of cotton squares is tested out of every batch received from the manufacturer. These cotton squares are subjected to the same analytical protocol as all other cotton samplers.

A laboratory control blank is run with every batch of cotton samplers. The control is subjected to the same analytical protocol as all other samplers.

System functioning tests of the analytical instruments are conducted in accordance with the manufacturer's recommendations.

All materials used in sampling and analysis work are routinely analyzed for the presence of any compounds that might create fluorescence peaks in or near the acceptable wavelength ranges for any of the tracer dyes. This testing typically includes approximately 1% of materials used.

### **Reports**

Reports are provided in accordance with the needs of the client. At a minimum we provide copies of the analysis graphs and a listing of stations and samples where dye was detected. The reports indicate both quantitative and qualitative results.

Work at the Ozark Underground Laboratory is directed by Mr. Thomas Aley. Mr. Aley has 40 years of professional experience in hydrology and hydrogeology. He is certified as a Professional Hydrogeologist (Certificate #179) by the American Institute of Hydrology. Mr. Aley has 35 years of professional experience in groundwater tracing with optical brighteners and other fluorescent tracing agents.