Application News

No.A494

Spectrophotometric Analysis

Simplified Measurement of Coumarin in Diesel Oil

Introduction

In Japan, diesel oil is subject to a consumption tax (national tax) and a diesel oil delivery tax (regional tax). However, kerosene and low-sulfur / high-sulfur A fuel oil are not subject to the delivery tax. Therefore, to avoid the tax, some vendors have been known to sell fraudulent diesel oil that has been mixed with kerosene or fuel oil. As a countermeasure, starting in March 1991, the then Ministry of Trade and Industry required addition of a 1 ppm concentration of coumarin to commercial kerosene and low-sulfur / high-sulfur A fuel oil products, so that they can be easily identified. Consequently, local tax bureaus have been using this marker for inspecting diesel oil by random sampling. If coumarin is detected, it means kerosene or low-sulfur / high-sulfur A fuel oil was mixed in with the diesel oil and legal measures or other actions are taken against the violator.
Therefore, on December 10, 2010, the Japan Petroleum Institute (Testing and Analysis sub-committee of the Product committee) established standard JPI-5S-71-2010 as the official method for analyzing coumarin. In this example, we used Method A of the standard to measure the fluorescence spectrum of coumarin.

Analytical Procedure

The procedure for analyzing coumarin is summarized below and a photograph of the RF-6000 spectrofluorophotometer used to identify the coumarin diesel oil marker substance is shown in Fig. 1. Equipment and reagents required for the analysis are listed in Table 1.

Analytical Procedure

(1) Prepare various solutions.
(2) Prepare a standard sample for creating a calibration curve.
(3) Prepare sample for quantitative analysis.
(4) Shake and isomerize (UV irradiation).
(5) Prepare calibration curve.
(6) Measure unknown sample.

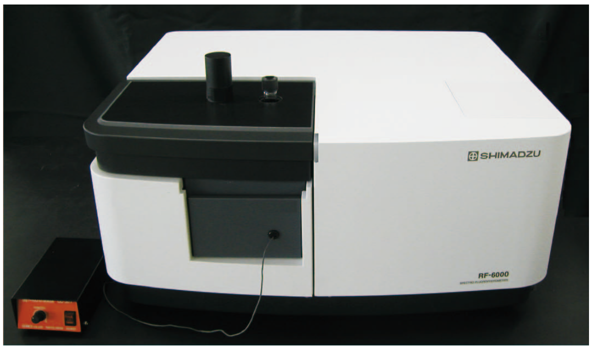

Fig. 1 RF-6000 Spectrofluorophotometer Coumarin (Diesel Oil Marker) Identification System

Table 1 Equipment and Reagents Required for Coumarin Analysis

(1)	RF-6000 spectrofluorophotometer system
(2)	Coumarin analysis kit (test tube holder with stirrer)
(3)	Dedicated coumarin measurement test tube (with stirrer)
(4)	Volumetric flasks (100 mL, 200 mL, and 500 mL)
(5)	Volumetric pipettes (1 mL, 2 mL, 5 mL, 6 mL, 8 mL, and 10 mL)
(6)	Measuring pipettes (0.5 mL, 1 mL, 2 mL, and 10 mL)
(7)	Test tube stand for 23 mm diameter tubes
(8)	Disposable gloves
(9)	Coumarin
(10)	Toluene
(11)	n-Dodecane
(12)	Sodium hydroxide and sodium nitrate for preparing alkaline aqueous solutions
(13)	1-Butanol and ethanol reagents for preparing alcohol solutions

Note: Items (9), (12), and (13) can be substituted with the Shimadzu RF Quantitation Reagent Kit.
A test tube shaker would also be helpful.

Preparing Solutions

Prepare each solution according to steps (a) to (e) below.

(a) Coumarin standard stock solution (10,000 mg/L)
(Can be stored for 3 months in a sealed container in a cool dark location)
Accurately weigh 1.0 ±0.005 g of coumarin into a 100 mL volumetric flask and fill to volume with toluene.

(b) Coumarin standard solution (100 mg/L)
Measure 5 mL of the coumarin standard stock solution (a) with a volmetric pipette and place it in a 500 mL volumetric flask. Then fill to volume with n-dodecane.

(c) Coumarin standard solution (1 mg/L)
Measure 5 mL of coumarin standard solution (b) with a volmetric pipette and place it in a 500 mL volumetric flask. Then fill to volume with n-dodecane.

(d) Alkaline aqueous solution (can be stored sealed for 1 month in a cool dark location)
Weigh 10 ±0.1 g sodium hydroxide and 20 ±0.1 g sodium nitrate and place them in a 100 mL volumetric flask. Then fill to volume with water.

(e) Alcohol solution (can be stored sealed for 1 month in a cool dark location)
Mix 80 mL 1-butanol and 60 mL ethanol.

Preparing Measurement Samples and Standard Samples for Creating a Calibration Curve

Insert stirrers in five test tubes used for creating the calibration curve. Then dispense the solutions indicated in Table 2. Prepare the measurement sample by inserting the stirrer in the test tube and then dispensing 1 mL of the measurement sample, 6 mL n-dodecane, 5 mL alkaline aqueous solution, and 8 mL alcohol solution.

Shaking and Isomerization

Install each test tube in the shaker and shake for three minutes at 240 rpm or faster. If a shaker is not available, shake by hand. Let stand for five minutes after shaking. Then confirm that the contents have separated into three layers, as shown in Fig. 2. From the top, these layers are the dodecane, alcohol solution, and alkaline aqueous solution layers.

Next, place the test tubes in the cell holder of the RF-6000 spectrofluorophotometer coumarin diesel oil marker identification system. Isomerize the coumarin by irradiating with 360 nm UV excitation wavelength (10 nm bandwidth) for three minutes while stirring with the stirrer. The isomerization progress can be checked by setting the fluorescence wavelength to 500 nm (10 nm bandwidth) and confirming the change in fluorescence intensity over time. Analytical conditions are indicated in Table 3. A time-course graph is shown in Fig. 3, with elapsed time on the horizontal axis and fluorescence intensity on the vertical axis. Irradiating samples with UV light causes the fluorescence intensity to increase with elapsed time. When the fluorescence intensity becomes constant isomerization is considered stabilized.

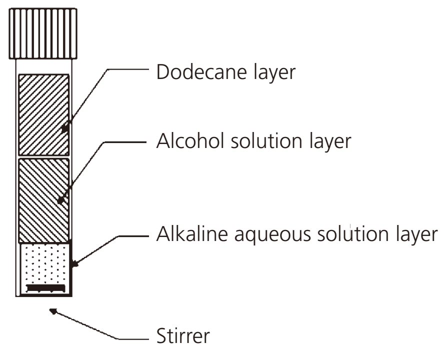

Fig. 2 Diagram of Test Tube Contents Separated into Three layers

Table 3 Analytical Conditions

Measurement mode	: Time course
Excitation wavelength	: 360 nm
Emission wavelength	: 500 nm
Bandwidth	: Ex: 10 nm, Em: 10 nm

Table 2 Preparing Standard Samples for Creating Calibration Curve

Types of Calibration Curves	Mixture Ratio (%)	0.0	10.0	40.0	80.0	120.0
	Coumarin Content (mg/L)	0.00	0.10	0.40	0.80	1.20
Reagent Acquisition Quantity (mL)	Coumarin Standard Solution (1.0 mg/L)	0	0.10	0.40	0.80	1.20
	n-Dodecane	7.0	6.9	6.6	6.2	5.8
	Alkaline Aqueous Solution	5.0	5.0	5.0	5.0	5.0
	Alcohol Solution	8.0	8.0	8.0	8.0	8.0

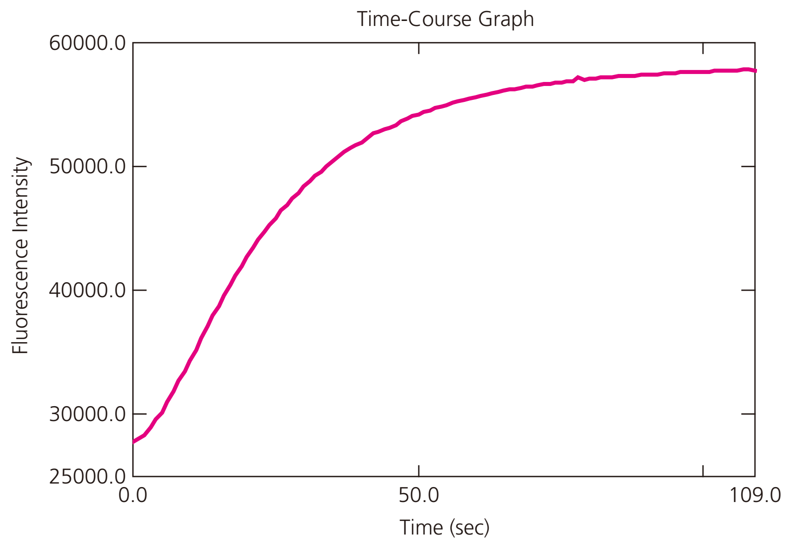

Fig. 3 Change in Fluorescence Intensity Due to Coumarin Isomerization

Isomerization Reaction of Coumarin

In an alkaline solution, coumarin breaks down by hydrolysis to form *cis*-*o*-hydroxycinnamic acid. If additionally irradiated with UV rays, it is isomerized to form *trans*-*o*-hydroxycinnamic acid. The structure of these isomers are shown in Fig. 4. When coumarin changes to *trans*-*o*-hydroxycinnamic acid, it emits fluorescent light. Coumarin can be quantitated by measuring the associated fluorescence intensity.

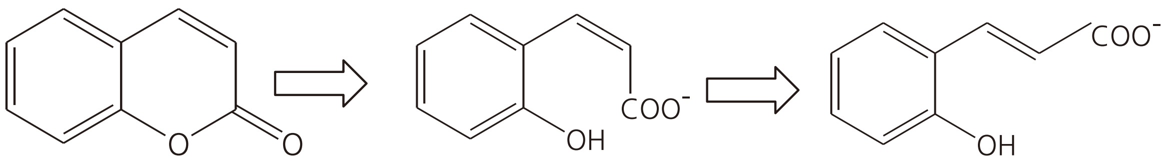

Fig. 4 Isomerization Reaction of Coumarin

Preparing a Calibration Curve and Measuring Coumarin Added to Diesel Oil

After irradiation with UV light, samples are measured using the analytical conditions indicated in Table 4. The fluorescence spectrum measured from the standard sample is shown in Fig. 5. The calibration curve is shown in Fig. 6. The squared correlation coefficient of the calibration curve, r^2, was 0.99965.

Results from measuring the measurement sample prepared by adding 0.5 ppm coumarin to commercial diesel oil are shown in Table 5. The quantitative results were approximately equivalent to the added quantity.

Table 4 Analytical Conditions

Excitation wavelength	: 360 nm
Emission wavelength	: 500 nm (390 to 630 nm when scanning spectra)
Bandwidth	: EX: 10 nm, EM: 10 nm

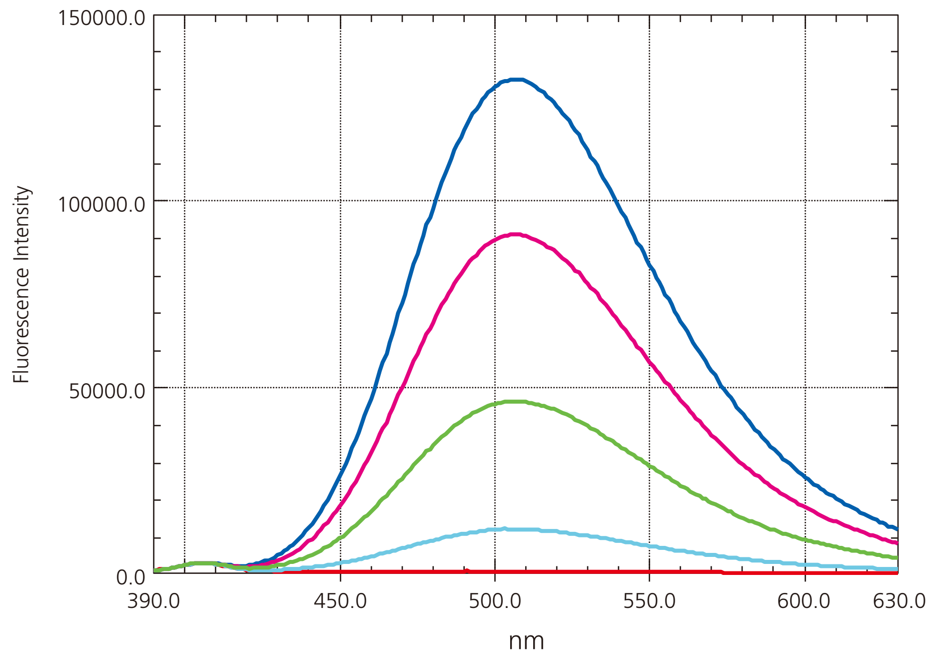

Fig. 5 Fluorescence Spectra of Standard Samples
In order of fluorescence intensity, with the highest intensity first, the corresponding concentrations are 1.2 ppm, 0.8 ppm, 0.4 ppm, 0.1 ppm, and 0 ppm.

	Sample Name	Concentration (ppm)	Fluorescence Intensity (500 nm)
1	0 ppm	0	470
2	0.1 ppm	0.1	11603
3	0.4 ppm	0.4	45767
4	0.8 ppm	0.8	90144
5	1.2 ppm	1.2	131583

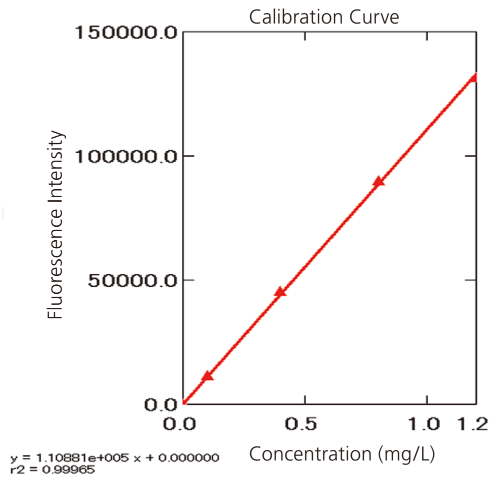

Fig. 6 Calibration Curve

Table 5 Measurement Results for Coumarin Added to Diesel Oil

Quantity Added (ppm)	Fluorescence Intensity	Measurement Result (ppm)
0.50	57440	0.514

Conclusion

This example showed that the Shimadzu RF-6000 spectrofluorophotometer can be used to easily and accurately measure coumarin according to Method A of the standard specified by the Japan Petroleum Institute.

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