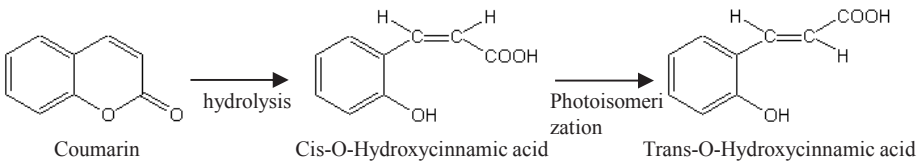
Application Note
FP-0002

High Sensitivity Coumarin Analysis

Introduction

To prevent the production of illegal light diesel oil, which contains kerosene or heavy oil, 1 ppm of coumarin is added to either the kerosene or a heavy oil as a discriminator. The analysis procedure for determining the discriminator and its mixing concentration is standardized by the National Petroleum Dealers Association and uses a spectrofluorometer to determine concentration.

Coumarin is hydrolyzed in alkaline solution and becomes cis-O-hydroxycinnamic acid. The cis-O-hydroxycinnamic acid is then isomerized by ultraviolet radiation and converted to trans-O- hydroxycinnamic acid. Since trans-O- hydroxycinnamic acid fluoresces, the product can be quantified using fluorescence spectroscopy.



Coumarin | Cis-O-Hydroxycinnamic acid | Trans-O-Hydroxycinnamic acid

Scheme 1. Hydrolysis and photoisomerization of coumarin.

While the current procedure is relatively simple, the purpose is to determine the coumarin concentration within more than a couple percent of the related oils. This application note introduces a high sensitivity system to improve the concentration detection limit to less than 1 percent and reduce the quantitation limit.

Keywords

FP-6300, Fluorescence, Materials, Filter set

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: http://www.jascoinc.com/applications

Experimental

1000 ppm of undiluted coumarin solution was made by dissolving 100 mg of coumarin in an aromatic solvent such as n-propyl benzene. The standard coumarin samples were prepared by diluting 100 µL of the undiluted coumarin solution using 1 ppm of n-dodecane and further diluting the solution to 0.1 ppm. For the alkaline solution reagent, 10 g of sodium hydroxide and 20 g of sodium nitrate were dissolved in 100 mL of Millipore water and kept in a polyethylene vessel. 40 mL of 1-butanol and 30 mL of ethanol were mixed for the alcohol solution. The standard samples were then mixed in test tubes, according to the specified ratios in Table 1. Following the procedure in Figure 2, the samples were shaken to hydrolyze the coumarin and then extracted in the alkaline solution. The samples were then kept stationary for 5 minutes to allow for separation of the solution layers. The photoisomerization reaction was induced by irradiating the sample using a spectrofluorometer and an excitation wavelength of 360 nm. The fluorescence intensity was detected at 500 nm and used to generate a calibration curve.

Additive Concentration	Coumarin Solution (mL)	n-dodecane (mL)	Alkaline Solution (mL)	Alcohol Dolution (ml)
0%	0	4.2	3	4.8
1%	0.06	4.14	3	4.8
2%	0.12	4.08	3	4.8
4%	0.24	3.96	3	4.8
6%	0.36	3.84	3	4.8
8%	0.48	3.72	3	4.8
10%	0.96	3.24	3	4.8

Table 1. Mixing ratios of the standard solution.

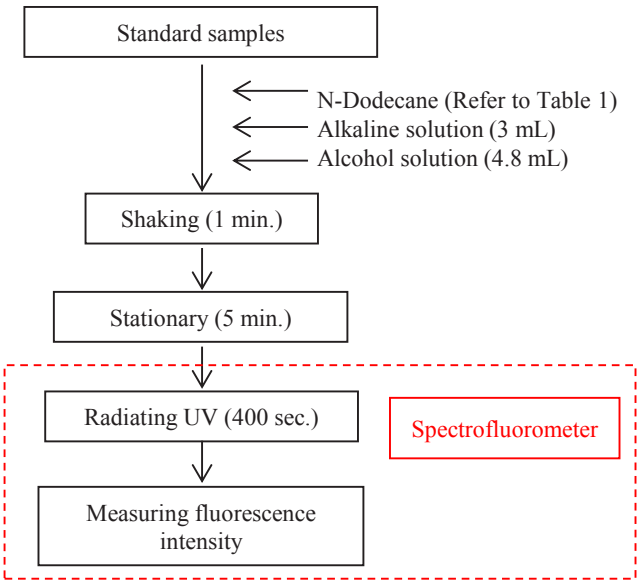


Figure 1. Flow chart of analysis procedure.

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: http://www.jascoinc.com/applications

Measurement Conditions			
Fluorescence		**Time Course**	
Excitation Wavelength	360 nm	Excitation Wavelength*	360 nm
Scan Speed	1000 nm/min	Emission Wavelength	500 nm
Excitation Bandwidth	10 nm	Excitation Bandwidth	20 nm
Emission Bandwidth	10 nm	Emission Bandwidth	10 nm
Data Interval	1 nm	Data Interval	2 sec
Response Time	Fast	Response Time	2 sec
Sensitivity	High	Sensitivity	High
Scan Speed	500 nm/min		

**The excitation was set at 20 nm to perform photoisomerization effectively for the Time course measurement. Excitation bandwidth was set at 10 nm for the Spectrum measurement in order to suppress reduction of fluorescence intensity due to photolysis.*

Results

The time course and spectral measurement data of the standard samples with varying additive concentrations are shown in Figures 2 and 3. Both figures indicate an increase in fluorescence intensity with increasing additive concentration and Figure 2 shows that the photoisomerization reaction is finished in 150 seconds after exposing the samples to UV radiation.

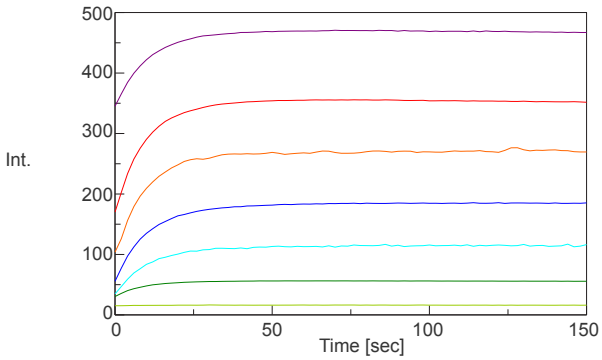


Figure 2. Time Course measurement of the photoisomerization of the standard samples varying additive concentrations.

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: http://www.jascoinc.com/applications

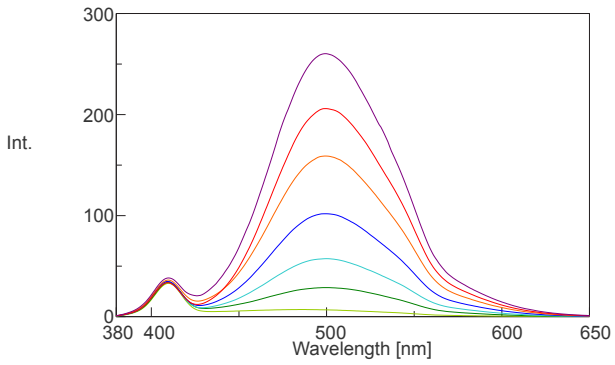


Figure 3. Fluorescence spectra after the photoisomerization reaction for varying additive concentrations.

A calibration curve plotting the fluorescence intensity at 500 nm as a function of the additive coumarin concentration is shown in Figure 4 and the corresponding values in Table 2. The correlation coefficient obtained for the calibration curve was 0.9993, indicating good linearity.

Additive Concentration	Fluorescence Intensity (at 500 nm)
0%	6.7077
1%	28.7548
2%	57.3873
4%	101.829
6%	158.903
8%	205.882
10%	260.236

Table 2. The additive coumarin concentrations with their corresponding fluorescence intensities at 500 nm.

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: http://www.jascoinc.com/applications

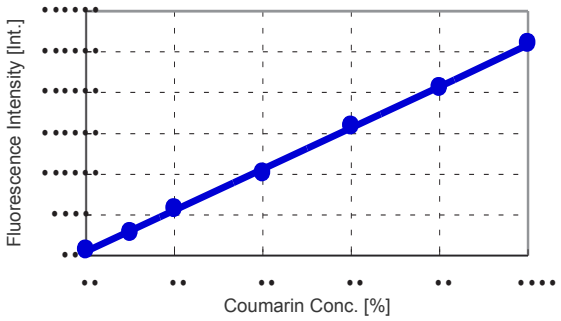


Figure 4. Calibration curve for the coumarin discriminator.

The fluorescence measurements of 0% and 1% standard solution concentrations were repeated 5 times and are shown in Figure 5. The standard deviation for the fluorescence intensity was 0.4357 while the coumarin concentration was 0.0172.

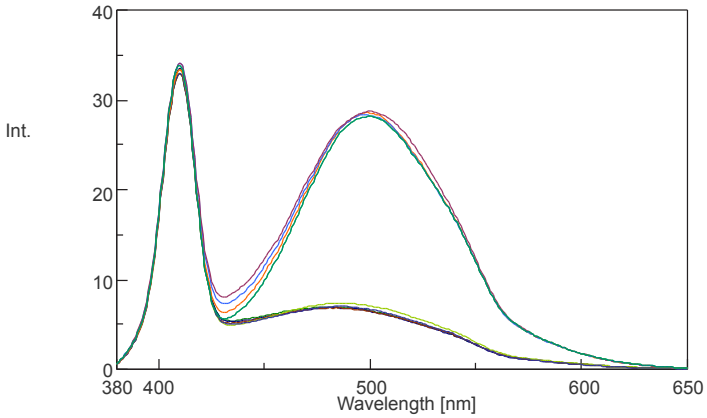


Figure 5. Fluorescence spectra of 0% and 1% standard sample solutions. 5 measurements were taken for each concentration.

Conclusion

These results demonstrate that it is possible to use the high sensitivity spectrofluorometer system to analysis diesel oil discriminators with a 0.06% detection limit and 0.2% quantitation limit*.

**Detection limit was calculated by 3 sigma and quantitation limit was calculated by 10 sigma.*

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: http://www.jascoinc.com/applications