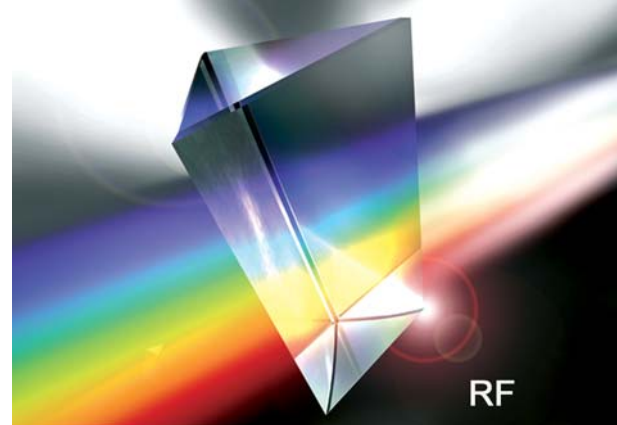


Application Note

Fluorescence library including Emission and Excitation Spectra from Pharmaceuticals and other materials



Libraries are well known for other analytical field like Infrared measurement technique, mass spectroscopy and NMR. All techniques have one thing in common that they generate under stable conditions a dataset possible to be used for identification. Fluorescence spectroscopy is a bit different. We have here two measurement techniques under one application field. The nature of the light itself contains effects which are not seen in the other identification techniques.

But nevertheless even having spectra and the description of spectra, fitting parameters and the view is a welcome help for any analysis.

Such a library is helpful for the quality control of a material or different materials, and to have some kind of identification possibility. The signals of the fluorescence are by nature broad bands but significant and selective for each substance under the criteria of fluorescence spectroscopy. Some substances have even under the condition of high resolution a characteristic signal shape like anthracene or salicylic acid.

In figure 1 emission and excitation spectra are shown from salicylic acid. They are different in shape by the nature of the measurement. Clear is to see with this comparison that the fluorescence (emission) is time delayed to the absorbance (excitation).

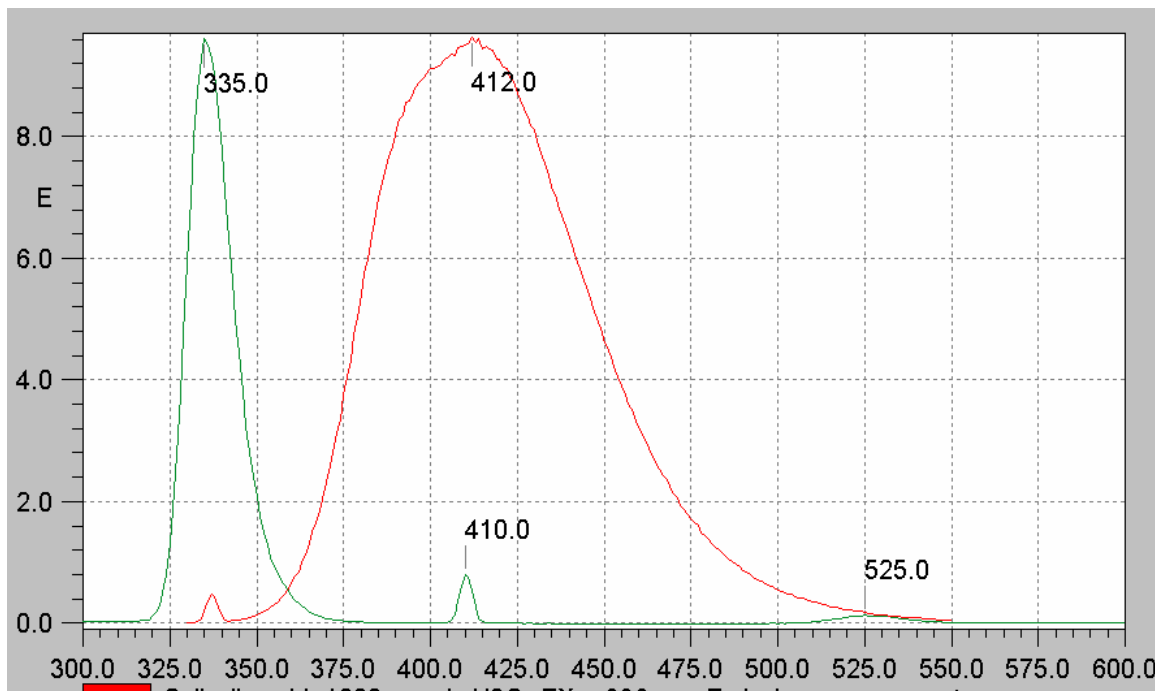


Fig. 1: Emission spectrum (red) and excitation spectrum (green) from salicylic acid, 1000ppm in H₂O

Ovalene Filter (Filter2)	H2O
EX = 341 nm, EM = 340 - 600 nm, EX-Slit = 3,0 nm, EM-Slit = 5,0 nm, Sensitivity: low, Emission measurement	Benzoic acid, 1000 ppm in H2O
Ovalene Filter (Filter2)	Benzoic acid, 1 ppm in H2O
EX = 200 - 600 nm, EM = 480 nm, EX-Slit = 3,0 nm, EM-Slit = 5,0 nm, Sensitivity: low, Excitation measurement	Benzene-1,3,5-tricarboxic acid, 1000 ppm in H2O
p-Terphenyl Filter (Filter3)	Benzene-1,3,5-tricarboxic acid, 1 ppm in H2O
Tetraphenylbutadiene (Filter4)	L-Tryptophan, 1000 ppm in H2O
Rhodamine B (Filter6)	L-Tryptophan, 10 ppm in H2O
Fluoresceine, 10 ppm in Ethanol	m-Cresol, 1000 ppm in 3,84%iger H2SO4 (Suspension!)
Fluoresceine, 1 ppm in Ethanol	Anthracene-9-carboxic acid, 1 ppm in Ethanol
Chininsulfate, 1000 ppm in 3,84%iger H2SO4	Anthracene-9-carboxic acid, 1000 ppm in Ethanol
Chininsulfate, 1 ppm in 0,004%iger H2SO4	DL-Phenylalanine, 2 ppm in H2O
Chininsulfate, 10 ppm in 0,04%iger H2SO4	DL-Phenylalanine, 1000 ppm in H2O
Salicylic acid, 1000 ppm in H2O	2-Methylindole, 2 ppm in Ethanol
Salicylic acid, 1 ppm in H2O	2-Methylindole, 1000 ppm in Ethanol
Alizarinsulfonic acid Sodiamsalt, 1 ppm in H2O	
Alizarinsulfonic acid Sodiamsalt, 1000 ppm in	

Table 1: Listing of substances which are content of library

At top of the list are two typical parameter sets, one for emission and the other for excitation measurement. Parts are also substance spectra in different concentrations and different sensitivity of the instrument which can be set from high to low and means approx. (factor 50 increase of intensity). For the library 23 substances were investigated. The intention of the measurement was to get a representative spectrum. It was not the intention to find all

selective excitation or emission spectra for each substance.

Benefit of such library is that it is possible to build up a home made one fitting to substances used in the company. Another thing is to search for text to get specific parameters of substance, or at minimum information of a spectrum with the help of the spectrum view.

The given specifications serve purely as technical information for the user. No guarantee is given on technical specification of the described product and/or procedures.

Instrument

- Shimadzu Fluorescence Spectrophotometer RF-5301PC
- 1 cm squared fluorescence quartz cell
- panorama Fluorescence software