

Standard Operating Procedure of Spectrofluorimetry

1- Purpose

This guideline describes the use of the JASCO FP-750 Spectrofluorimetry made in Japan with an identification number of 18 located in the Pharmaceutical Analysis Laboratory of the Pharmaceutical Analysis Research Center.

2- Scope

This method is used to measure pharmaceutical and non-pharmaceutical compounds that have fluorescence.

3- Responsibility

The laboratory assistance is responsible for the accuracy of the apparatus.

4- Materials and equipment:

4-1) equipment

Name: Spectrofluorimetry

4-2) Materials

Distilled water and Rhodamine 6g

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5- Guidelines for use

- *Plug the apparatus into a power outlet.*
- *Make sure that the cuvette is not inside the cell holder and its lid is closed.*
- *Turn on the device with a key on the back of the device.*
- *Give the device 5 minutes to warm up.*
- *Run the device software called Spectra manager.*
- *In the opened window, select the “Spectrum measurement” and run it.*
- *Then, in the opened window, select the “Parameters” from the “Measurement” option and fill the following options according to the desired compounds.*

Measurement mode → Emission

Excitation band width

Emission band width

Sensitivity

Excitation wavelength

Start

End

Press the “Okey” key to save the selected conditions.

- *Then select “Auto Zero” from the “Measurement” section.*
- *From the “Measurement” section, open the “Shutter” option and open the “Excitation shutter” and “Emission shutter”.*

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- *Fill the cuvettes with the the analyte solution and place them in the cell holders.*
- *In the opened “Spectrum measurement” window, click on the “Start” key to draw the spectrum for the analyte.*
- *To save the data, in the left column of the “Spectra manager” window, select the “Spectra analysis” option and from the “File” section, select “Save as” where you can save the spectrum in either JWS or TXT format.*
- *To turn off the device, exit from the software, press the power button and unplug the device.*

6- Warning and safety precautions

- *Connect the device to the power stabilizer to prevent serious damage to the device due to power fluctuations.*
- *Avoid handing over the device to beginners and unfamiliar with Spectrofluorimetry principles.*
- *When operating with the cuvette, ensure that the inside and outer walls of the cuvette are clean.*
- *Avoid placing the cuvette inside the cell holder after the work is completed.*
- *Avoid pouring any solution into the cell holder.*
- *After work is done, it is recommended that the device is unplugged and covered.*
- *Since the spectrofluorimeter is a precise and sensitive instrument, do not replace the device parts without the approval of the relevant agency.*

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7- Maintenance

The spectrofluorimeter should be kept dry and away from direct sunlight. After work is done, turn off the device and cover it.

8- Quality control

The spectrofluorimeter should be qualitatively controlled for 6-month periods in terms of linearity control, slit width accuracy and wavelength accuracy.

Deionized water is used for checking the wavelength accuracy. One wavelength selector is fixed at a wavelength of interest and the other is scanned. The water Raman peak appears at a wavelength that is about 3400 cm^{-1} lower in energy than the excitation wavelength. The Raman scattering intensity is proportional to λ^{-4} .

To check the linearity of the detection system, it uses a set of solutions obtained by serial dilution of a fluorescent stock solution. Users must ensure that the fluorescence intensities of samples are reproducible and do not decrease over the time period that they are being excited and measured.

For spectral correction of detection system responsivity, it is used from Certified Reference Materials (CRM, usually Rhodamine). For CRMs, the relative intensity and uncertainty values are given as a function of emission wavelength at a fixed excitation wavelength. A solution of a known concentration of CRM is prepared and its spectrum is collected and compared to the certified spectrum according to the instructions.

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9- Fluorescent compounds

Class	Compounds	λ_{ex} (nm)	λ_{em} (nm)
Amino acids	Phenylalanine	240	282
	Tyrosine	260	303
	Tryptophan	280	348
Vitamins	Vitamin B 2	500	550
	Vitamin B 12	250	337
	Folic acid	348	450
Catecholamines	Dopamine	279	315
	Norepinephrine	280	317
	Methyl dopa	280	317
	Resorcinol	278	310
Pharmaceuticals	Quinine	250, 350	450
	Salicylic acid	299	409
	Codeine	215	350
	Verapamil	280	310
	Caffeine	270	330
	5-Aminosalicylic acid	340	500
	Amoxicillin	255	400
	Cetirizine	230	297

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	<i>Naproxen</i>	330	355
	<i>Metoprolol</i>	276	296
	<i>Amiloride</i>	362	415
	<i>Celecoxib</i>	267	360
	<i>Ibuprofen</i>	226	290
	<i>Dextromethorphan</i>	277	588
<i>Metabolites</i>	<i>1-Hydroxynapthalene</i>	277	355
	<i>2-Hydroxynapthalene</i>	277	355
	<i>2-Hydroxyfluorene</i>	270	327
	<i>1-Hydroxyphenanthrene</i>	256	370
	<i>2-Hydroxyphenanthrene</i>	256	370
	<i>3-Hydroxyphenanthrene</i>	256	370
	<i>4-Hydroxyphenanthrene</i>	256	370
	<i>9-Hydroxyphenanthrene</i>	256	370
	<i>3-Hydroxyfluoranthene</i>	292	473
<i>Environmental pollutants</i>	<i>Pyrene</i>	320	470
	<i>Benzo [a] pyrene</i>	280	405, 430
<i>Miscellaneous</i>	<i>Terbium ion</i>	295	490, 545

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10- References

- *Catalog and Instructions given by Manufacturer*
- *DeRose, P.C., 2007. Standard guide to fluorescence: Instrument calibration and validation. US Department of Commerce, Technology Administration, National Institute of Standards and Technology.*

<i>Document ID PARC 4</i>	
<i>Document name</i>	<i>Standard Operating Procedure of Spectrofluorimetry</i>
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