A Review on First Derivative Synchronous Spectrofluorimetry

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Abstract:

The review article deals with the principles and the applications of advanced fluorimetric Technique that is First Derivative Synchronous Spectro-fluorimetry (FDSF) and advantages over conventional Spectro-fluorimetry. Fluorescence is the most common and useful type of photoluminescence in the analytical chemistry. In conventional fluorimetric methods, a high sensitivity and selectivity are generally expected but problems of selectivity are seen in Multi-component Analysis because of overlapping of spectra. The combination of derivative and synchronous fluorescence spectroscopy improves selectivity, Spectral discrimination and decrease the effects of background matrices. Synchronous fluorescence spectroscopy techniques are classified according to different scanning modes of monochromators into constant wavelength difference, variety angle, constant energy difference. The main advantages are selectivity, economic, simplicity, and rapidity. This method has wide applications in clinical and multi-component analysis.

Key words: First Derivative Synchronous Spectrofluorimetry (FDSF), Zero Order, Second Derivative, Fluorescence Spectroscopy.

Introduction

Spectrofluorimetric methods have found more selective than normal UV-spectroscopy due to quantification of substance at specific excitation and emission wavelengths. Derivative Spectrofluorimetry/ spectrophotometry provides a greater selectivity and spectral discrimination than normal techniques. It is the powerful approach for resolution of one analyte whose peak is hidden by a large overlapping peak of another analyte in multi-component analysis. The fusion of scanning Spectrofluorimetry with derivative techniques is advantageous in terms of sensitivity, spectral discrimination and more reliable identification of chemical species in multi component analysis (1).

Being simple, highly selective and low interference are the advantages of synchronous fluorescence spectroscopy [SFS] over conventional fluorescence spectroscopic methods. SFS is a very simple and efficient process for quantitative estimation, as a consequence of its sharp and narrow spectrum The amplitude of derivative signal is inversely proportional to band width of the original spectrum, subsequently combination of SFS and differentiation is further advantageous than traditional emission spectrum. Derivative synchronous fluorometry (DSF) has become one of the buzzwords in the field of analytical chemistry (2). Fluorescence detection techniques are commonly used in modern biochemical research and disease diagnosis due to their high sensitivity and specificity; however, conventional fluorescence techniques suffer from emission isotropy, leading to challenges like low collection efficiency and background interference (3).

Fluorescence Spectroscopy:

Fluorescence spectroscopy is a type of electromagnetic spectroscopy analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in, molecules of certain compounds and causes them to emit light. A complementary technique is absorption spectroscopy. Devices that measure fluorescence are called as Fluorimeter. A molecule can be excited from its ground electronic state to an electronic excited state by absorbing energy in the form of visible or ultraviolet light. Many molecules are capable of emitting this energy as radiation, thus returning to the ground state. The emitted radiation is called as fluorescence. Fluorescence occurs in simple as well as in complex gaseous,

liquid and solid chemical systems. In atomic species, the absorbed radiation is reemitted without a change in frequency is known as resonance fluorescence or resonance radiation. Many molecular species also exhibit resonance fluorescence. Much more often, however, molecular fluorescence bands center at wavelengths longer than the resonance line. This shift towards longer wavelength is termed as Stokes shift.

Theory of Fluorescence

Molecules have various states referred to as energy levels. Fluorescence spectroscopy is primarily concerned with electronic and vibrational states. Generally, the species being examined has a ground electronic state (a low energy state) of interest, and an excited electronic state of higher energy. Within each of these electronic states are various vibrational states. In fluorescence spectroscopy, the species is first excited, by absorbing a photons, from its ground electronic state to one of the various vibrational states in the excited molecule to lose vibrational energy until it reaches the lowest vibrational state of the excited electronic state. The molecule then drops down to one of the various vibrational levels of the ground electronic state again, emitting a photon in the process. As molecules may drop down into any of several vibrational levels in the ground state, the emitted photons will have different energies, and thus frequencies. Therefore, by analysing the different frequencies of light emitted in fluorescent spectroscopy, along with their relative intensities, the structure of the different vibrational levels can be determined.

The different wavelengths of fluorescent light emitted by a sample are measured using a monochromator, holding the excitation light at a constant wavelength. This is called an emission spectrum. An excitation spectrum is the opposite, whereby the emission light is held at a constant wavelength, and the excitation light is scanned through many different wavelengths. An emission map is measured by recording the emission spectra resulting from a range of excitation wavelengths and combining them all together.

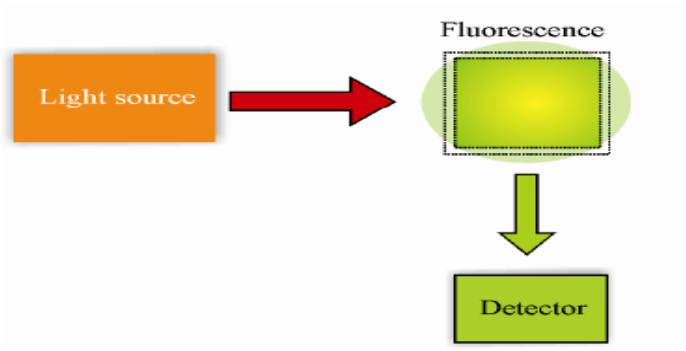

Fig 1.0: Essential components of a fluorescence spectrometer

Synchronous Fluorescence:

The ability to analyse complex multi-component mixtures without resorting to tedious separation procedures is extremely useful for routine analysis. Single-wavelength fluorescence measurement is limited in its ability to analyse complicated multi-component samples when they have severely overlapping emission and/or excitation spectra. This can be overcome by using synchronous fluorescence scan (SFS), where overlapping of spectra can be minimized. The selectivity of SFS can still be increased by taking derivative spectrum, applying different multi-variate methods, selective fluorescence quenching. three-dimensional synchronous measurement or using some of these procedures in combination. Conventionally, chemical analysis is done in a laboratory using gravimetric, spectrometric, chromatographic and electro-analytical techniques after precipitation, separation and extraction procedures, in which large amounts of solvent and time are consumed. Moreover, contamination at the sampling stage and during handling cannot be avoided by separation and extraction procedures. Some of the instruments used for these procedures are expensive. Therefore, with the help of powerful computation and new analytical techniques, efforts are being made to develop analytical methods for continuous sensing of analytes in real time and *in situ*.

Despite the ability to select both the excitation and the emission wavelengths, conventional fluorescence methods have limited practical applicability. As most spectra of complex mixtures often cannot be resolved satisfactorily, they need a pre-separation procedure before analysis of a sample mixture. However, this can be overcome by using special techniques, such as synchronous fluorescence scan (SFS).

Synchronous Fluorescence Scan (SFS)

In conventional fluorescence, an emission spectrum is obtained by scanning the emission monochromator at various emission wave- lengths, λem, (SFS). At a particular excitation wavelength, λex; an excitation spectrum is obtained by scanning the excitation monochromator at various excitation wavelengths keeping the emission monochromator constant at a particular wavelength. The other possibility is to scan both the monochromators simultaneously, which is called synchronous fluorescence scan or spectroscopy

Depending on the scan rate three basic types of SFS technique are possible:

- ✓ If the scan rate is constant for both the monochromators and therefore a constant wavelength interval, λ, is kept between λem and λex, the technique is known as constant-wavelength SFS. This technique is very simple and is the most frequently used of all synchronous modes. Therefore, in general, SFS refers to this technique.

- ✓ The excitation and emission wavelengths may be varied simultaneously in such a manner that a constant frequency difference n, is maintained between them. This technique, constant-energy SFS, has not been used much.

- ✓ The excitation and emission wavelengths may be varied simultaneously but at different rates. These different rates allow the construction of planes at angles between 45 and 90 to the excitation x-axis throughout the whole spectrum. Known variously as variable-angle synchronous fluorimetry, variable separation synchronous fluorimetry or variable off-set synchronous fluorimetry, this technique also has not been used in many applications.

Derivative Spectroscopy

Derivative spectroscopy involves taking the first or higher derivatives of absorbance with respect to wavelength for qualitative analysis and for quantification. The concept of derivatizing spectral data was first introduced in the 1950s, when it was shown to have many advantages. However, the technique received little attention primarily because of the complexity of generating derivative spectra using early UV-Visible spectrophotometers. The introduction of microcomputers in the late 1970s made it generally practicable to use mathematical methods to

generate derivative spectra quickly, easily and reproducibly. This significantly increased the use of the derivative technique. Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. The transformations that occur in the derivative spectrum are understood by reference to a gaussian band which represents an ideal absorption band. If a spectrum is expressed as absorbance, *A*, as a function of wavelength, λ, and the derivative spectra are:

Zero order: In the context of derivative spectrophotometry, the normal absorption spectrum is referred to the fundamental, zero-order (D^o) spectrum.

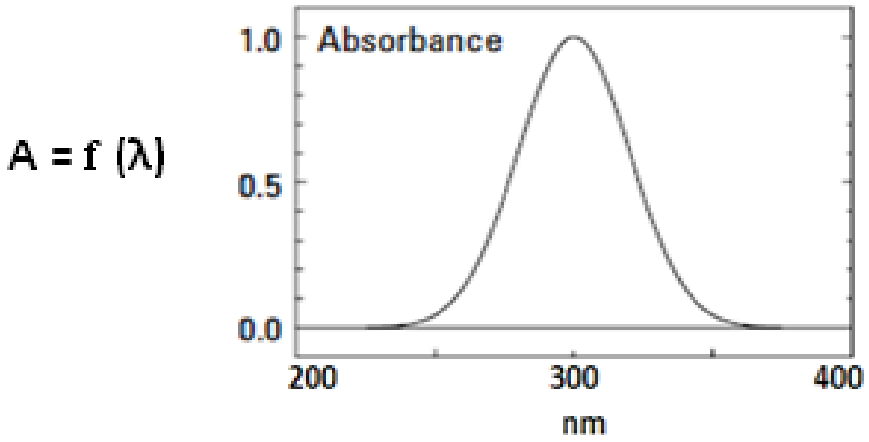

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Fig 2.0 : Zero-order UV-absorption spectrum

First order: A first-order (D^1) derivative is the rate of change of absorbance with respect to wavelength. A first-order derivative starts and finishes at zero. It also passes through zero at the same wavelength as λ_{max} of the absorbance band. Either side of this point is positive and negative bands with maximum and minimum at the same wavelengths as the inflection points in the absorbance band. This bipolar function is characteristic of all odd-order derivatives.

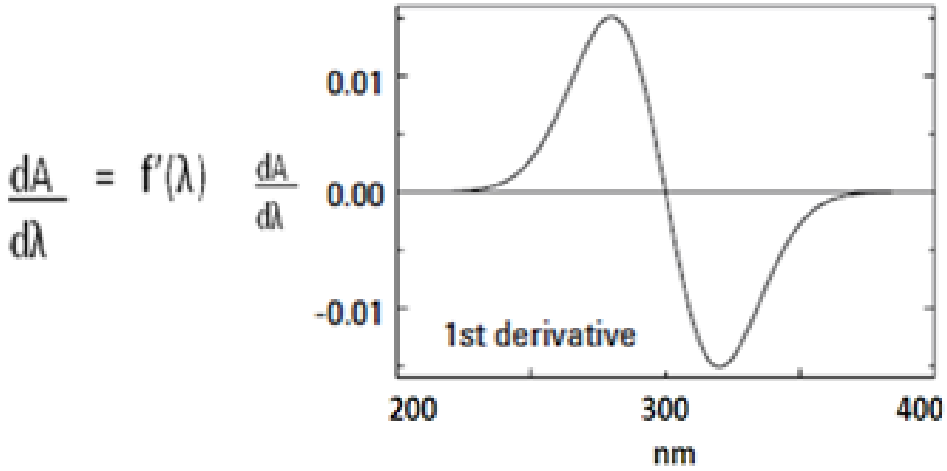

Fig 3.0 : First-order UV derivative spectrum

Second order: The second derivative spectrum is a plot of the curvature of the D^0 spectrum against wavelength. The most characteristic feature of a (D^2) derivative is a negative band with minimum at the same λ as the maximum on the zero-order band. It also shows two additional positive satellite bands either side of the main band. A fourth-order derivative shows a positive band. A strong negative or positive band with minimum or maximum at the same wavelength as λ_{max} of the absorbance band is characteristic of the even-order derivatives.

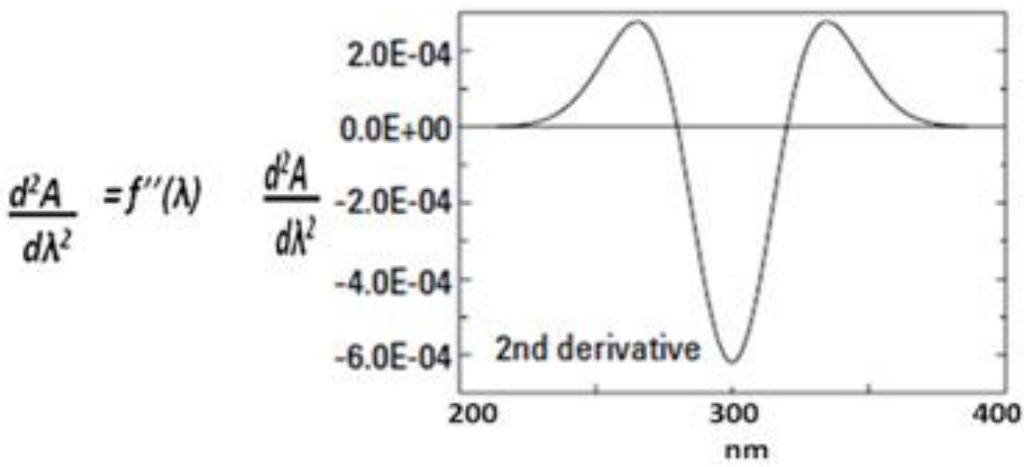

Fig. 4.0: Second-order UV derivative spectrum

Advantages of derivative spectroscopy

1. Precise determination of the wavelength of peak maxima can be obtained from the zero crossing point of the first derivative.

2 Spectral discrimination, as a qualitative fingerprinting technique to accentuate small structural differences between nearly identical spectra

3 Resolution enhancement, as a technique for increasing the apparent resolution of overlapping spectral bands in order to more easily determine the number of bands and their wavelengths.

4 Quantitative analysis, as a technique for the correction for irrelevant background absorption and as a way to facilitate multicomponent analysis

Analytical performance data of various drugs using first derivative synchronus spectrofluorimetry

S.no	Title of the Article	Drug	Method Valiadation
1	First derivative synchronous spectrofluorimetric method for the simultaneous determination of tramadol and celecoxib in their dosage forms and human plasma (4).	• Tramadol HCl	Excitation λ_{max} :230.2 nm Emission λ_{max} :282nm Linear range:0.15-0.50 LOD :0.011 µg/mL LOQ :0.032µg/mL
		• Celecoxib	Excitation λ_{max} :288.24 nm Emission λ_{max} :368 nm Linear range:0.50-5.0 LOD :0.088µg/ml LOQ :0.0266µg/ml
2	First derivative synchronous spectrofluorimetric analysis of	• Bisoprolol	Excitation λ_{max} : 232nm Emission λ_{max} :244.40nm

	bisoprolol fumarate and ivabradine in pharmaceutical and biological matrices. Investigation of the method greenness (5).	Fumarate • Ivabradine	Linear range:30.0-180.0 ng/ml Detection limits:5.28ng/ml Excitation $\lambda_{max:287nm}$ Emission $\lambda_{max:}$ 234.4nm Linear range:30.0-200.0ng/ml Detection limits:4.88ng/ml
3	First Derivative Synchronous Spectrofluorimetric Quantification of Telmisartan /Amlodipine Besylate Combination in Tablets (6).	• Amlodipine besylate • Telmisaratan	Excitation $\lambda_{max:355nm}$ Emission $\lambda_{max:}$ 458nm Linear range:1-6µg/ml LOD:0.013µg/ml LOQ:0.044µg/ml Excitation $\lambda_{max:358nm}$ Emission $\lambda_{max:}$ 675nm Linear range:4-14µg/ml LOD:0.070µg/ml LOQ:0.233µg/ml

4	Zero-crossing point derivative simultaneous spectrofluorimetric method for quantification of Nebivolol hydrochloride and Valsartan combination in tablets (7).	• Nebivolol HCl • Valsartan	Excitation λ max :272.2nm Emission λ max: 294nm Linear range:0.1-2.1 LOD: 0.016µg/ml LOQ:0.049µg/ml Excitation λ max :259nm Emission λ max:404nm Linear range :0.1-2.1 LOD:0.015µg/ml LOQ:0.047µg/ml
5	Simultaneous quantification of nebivolol hydrochloride and hydrochlorothiazide by first derivative UV-Spectroscopy (8).	• Nebiovol HCl • Hydrchlorothiazide	Excitation λ max :272.2nm Emission λ max:272.2nm Linear range :2-32 µg/ml LOD:0.230 /ml LOQ:0.769µg/ml Excitation λ max :272.0nm Emission λ max:280nm Linear range :4-24 µg/ml LOD:0.127µg/ml LOQ:0.425µg/ml

Conclusion

In the present study, a new simple, sensitive and time saving first derivative synchronous spectrofluorimetric method has been developed for simultaneous quantification of Tramadol HCL-Celecoxib, Bisoprolol fumarate-Ivabradine, Amlodipine besylate-Telmisaratan, Nebivolol HCL-Valsartan, Nebivolol HCL-Hydrochlorothiazide, and in binary mixture and pharmaceutical dosage forms. This spectrofluorimetric method has been found to be superior, because of its

highly specific spectral discrimination, readily available solvent, economical, eco-friendly and lack of extraction procedure. The assay values were in good concurrence with their respective labeled claim, which suggested no interference of formulation excipients in the estimation and the obtained results from validation proved the proposed method to be scientifically sound.

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