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Simultaneous Determination of Naproxen and Diflunisal using Synchronous Luminescence Spectrometry

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Abstract Binary mixtures of naproxen and diflunisal can be resolved by using zero-crossing first derivative emission spectrofluorimetry, first derivative constant wavelength synchronous luminescence spectrometry and first derivative constant energy synchronous luminescence spectrometry. These methods do not require any previous separation steps. The lowest quantitation limits for both drugs were obtained with first derivative constant wavelength synchronous luminescence spectrometry (0.002 and 0.015 µg ml^{-1} for naproxen and diflunisal, respectively). The measurements were performed in 40% methanolic aqueous medium at pH 8.0 provided by adding 0.02 M phosphate buffer solution. The proposed methods were successfully applied to the simultaneous determination of naproxen and diflunisal in pharmaceuticals and human serum samples with high precision and accuracy. Linearity, accuracy, precision, limits of detection, limits of quantitation, and other aspects of analytical validation are included in the text.

Keywords First derivative emission spectrofluorimetry · First derivative synchronous luminescence spectrometry · Zero-crossing technique-Naproxen and diflunisal · Pharmaceuticals · Human serum

Introduction

Fluorescence spectrometry is widely used in quantitative analysis because of its great sensitivity and selectivity as well as its relatively low cost. The technique has not, however, been widely applied to the simultaneous determination of several fluorescent components in mixtures, mainly because the fluorescence spectra of individual substances contain broad bands which may easily overlap. Generally, these compounds are determined by using a prior separation step which is rather time-consuming for routine analysis and in some cases requires special, expensive instrumentation. For this reason, the development of techniques allowing the direct determination of related compounds through careful selection of instrumental variables is of great interest. Among these techniques, synchronous and derivative fluorescence spectrometry are the most popular [1, 2].

Methodology and applicability of synchronous luminescence spectrometry has been previously discussed by Vo-Dinh [3]. This technique involves the simultaneous scanning of both the excitation and emission monochromators, synchronized in such a way that a well-defined relationship is maintained between the wavelengths of the monochromators. Conventionally, this relationship is a constant wavelength difference, $\Delta\lambda$, which is called constant wavelength synchronous luminescence spectrometry. When a constant energy difference is maintained between the monochromators, the technique is called constant energy synchronous luminescence spectrometry [1–3]. The advantages of synchronous techniques include a reduction in spectral complexity, in peak bandwidths, in Rayleigh scattering and even a reduction in Raman scattering [1, 2]. Synchronous fluorimetry is of great value in both qualitative [4] and quantitative analysis [5].

The application of derivative techniques to luminescence spectrometry was of great advantage [1, 2]. Differentiation narrows spectral bandwidths and enhances minor spectral features, thus improving the selectivity of multi-component

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spectra. Later on, many studies appeared where synchronous luminescence spectrometry and the derivative technique were combined to resolve mixtures with overlapping spectra [1, 2, 6, 7]. In this paper first derivative luminescence spectrofluorimetry, first derivative constant wavelength synchronous luminescence spectrometry and first derivative constant energy synchronous luminescence spectrometry were applied to resolve binary mixtures of naproxen and diflunisal, since they showed overlapping spectra.

Naproxen [NAP: (+)-2-(6-methoxy-2-naphthyl) propionic acid] and diflunisal [DIF: 2-hydroxy-5-(2, 4-difluorophenyl) benzoic acid] are non-steroidal anti-inflammatory drugs, which have primarily been used as analgesics. Binary drug mixtures containing NAP and DFL are increasingly used in the therapy of a variety of diseases [8]. The simultaneous control of these drugs in biological fluids may present some difficulties due mainly to their similar analytical properties and the interference from the matrix. Gas–liquid chromatography [9, 10], high-performance liquid chromatography [11, 12] and capillary electrophoresis [13] are the recommended techniques for the analysis of these mixtures, although most of these procedures are time-consuming and require sample pretreatment prior to measurement. In dosage forms, densitometric analysis [14] and capillary electrophoresis [13] have been reported for the simultaneous determination of both drugs.

Several fluorimetric methods have been applied to the individual determination of NAP and DIF based on their intrinsic fluorescence [15–18] or on derivatization reactions [16, 18, 19]. Synchronous fluorescence spectrometry was previously applied to analyze mixtures of salicylic acid and NAP [20] or DFL [21, 22]. The resolution of these binary mixtures were accomplished using partial least squares calibration [21], derivative synchronous fluorimetry [20] or derivative matrix isopotential synchronous fluorescence spectrometry [22]. However, only one report has been found concerning the simultaneous spectrofluorimetric determination of such combination. It is based on the intrinsic fluorescence of both compounds in sodium dodecylsulfate micellar medium using constant wavelength synchronous fluorescence spectrometry for the determination of NAP and DIF, in spiked serum samples [23]. To our knowledge, no reports are available concerning the spectrofluorimetric determination of such binary mixtures in their pharmaceutical formulations. Therefore, the development of simple spectrofluorimetric methods allowing the accurate determination of NAP and DIF in their pharmaceutical preparations and biological fluids could be considered of clinical value.

This paper describes three methods to resolve mixtures of NAP and DIF using their native fluorescence in methanolic aqueous solutions by employing first derivative emission spectra and first derivative synchronous fluorimetry (constant wavelength and constant energy). In all cases, the mixtures were resolved by scanning only one spectrum and only aqueous methanolic solutions were prepared without using any surfactant with its frothing problems. These are considered important advantages over the previously published work [23]. The method is as sensitive as or even more sensitive, in some cases, than the previously reported one [23] but owing to its simplicity and greater specificity, it can be easily applied to routine analysis and quality control studies of this mixture in addition to clinical or pharmacokinetic studies.

Experimental

Instrumentation

Fluorescence measurements were performed on a Perkin-Elmer LS-45 Luminescence spectrometer equipped with Xenon Lamp, connected to an Attaio S 3000 ST 386 computer. Data acquisition and data analysis were performed with Perkin-Elmer F. L. WINLAB software (version 4.00). To provide a suitable signal–noise ratio a scan speed of 240 nm/min and a width factor of 10 were selected throughout the measurements.

A Schott-Gerate pH meter Model CG 710 calibrated daily with standard buffers at room temperature was used.

Materials and reagents

Standards of NAP and DFL were kindly supplied by Rameda, The Tenth of Ramadan Co. (6th of October City, A.R.E). All reagents used were of analytical grade, namely: methanol (Panreac Co., E.U.), disodium hydrogenphosphate, phosphoric acid and sodium hydroxide. The water used was double glass distilled.

Phosphate buffer solutions (0.02 M) of different pH values were tried. Dilutions were made throughout the work using the mixing solvent (MS) which consists of 0.02 M phosphate buffer pH 8.0 and methanol (60:40).

(Maxipan)® tablets labeled to contain 200 mg NAP and 200 mg DFL (Rameda, 6th of October City, A.R.E) were used for the study.

Standard solutions and calibration graphs

Stock solutions of 50.0 μg/ml of NAP and DFL were prepared in methanol. These stock solutions were further diluted with MS to obtain working standard solutions of suitable concentrations (corresponding to the linearity range stated in Table 1). A reagent blank was prepared in a similar way.

Table 1 Regression and statistical parameters for the determination of NAP and DFL by the proposed spectrofluorimetric methods

Type of linearity	Linearity range (μg/ml)	LOD	r	$S_{y/x}$	a	S_a	b	S_b	F	Significance F
1 First derivative of emission spectra										
NAP	0.02–1.00	0.015	0.9995	0.2847	0.1868	0.1649	22.5747	0.3509	4,138.605	3.50×10^{-7}
DFL	0.02–1.00	0.014	0.9992	0.2114	0.0925	0.1224	−12.9276	0.2605	2,462.977	9.86×10^{-6}
2 First derivative of constant wavelength synchronous spectra										
NAP (Δλ=165 nm)	0.01–0.80	0.007	0.9997	0.4806	0.0225	0.2913	−73.5593	1.0138	5,265.151	5.77×10^{-6}
NAP (Δλ=122 nm)	0.002–0.04	0.0013	0.9998	0.4143	−0.093	0.4346	−1,034.700	13.1025	6,236.165	4.48×10^{-6}
DFL (Δλ=165 nm)	0.02–1.00	0.013	0.9997	0.1949	0.0131	0.1128	−19.9642	0.2402	6,909.149	1.26×10^{-7}
DFL (Δλ=202 nm)	0.015–1.00	0.010	0.9997	0.6914	0.6924	0.4004	−67.2177	0.8522	6,222.383	1.55×10^{-7}
3 First derivative of constant energy synchronous spectra										
NAP	0.02–1.00	0.011	0.9998	0.6231	0.0358	0.3436	66.3911	0.7371	8,110.98	9.11×10^{-8}
DFL	0.03–1.00	0.028	0.9995	0.3577	−0.0522	0.2072	−26.7732	0.4408	3,687.693	4.40×10^{-7}

LOD = limit of detection, r = correlation coefficient, $S_{y/x}$ = standard deviation of residuals, a = intercept, b = slope, S_a = standard deviation of intercept, S_b = standard deviation of slope, F = variance ratio, equals the mean of squares due to regression divided by the mean of squares about regression (due to residuals)

Preparation of laboratory-made mixtures

Accurate volumes of each of NAP and DFL stock solutions were transferred into 10-ml volumetric flasks and diluted to volume with MS to prepare six mixtures within the concentration range of each compound (Table 2).

Preparation of tablet solutions

Ten Maxipan® tablets were weighed and powdered, after removing the outer orange-colored coat with a cotton swab wetted with water. Accurate weight of the powder equivalent to 5.0 mg of each of NAP and DFL was extracted with 60 ml methanol in an ultrasonic bath for 20 min then filtered. The filterate was collected in 100-ml volumetric flask and diluted to volume with methanol. Portions of 0.5 ml of the previously prepared solution were diluted with MS in 50 ml volumetric flasks to prepare tablet solutions containing 0.5 μg ml^{-1} NAP and 0.5 μg ml^{-1} DFL.

Preparation of serum samples

To 0.5 ml of human serum containing 5–40 μg ml^{-1} of both NAP and DFL, 2.0 ml methanol was added. The mixture was sonicated for 5 min then centrifuged for 15 min at 450×g. A volume of 0.2 ml of the clear supernatant solution was diluted with MS to 10.0 ml.

Table 2 Evaluation of the precision and accuracy for the determination of NAP and DFL in laboratory-made mixtures by the proposed spectrofluorimetric methods

Ratio of mixture components	% Recovery					
NAP:DFL	NAP			DFL		
	I	II	III	I	II	III
1.0:1.0	101.3	100.7	98.7	100.6	99.8	101.4
1.0:1.5	100.5	100.5	99.4	99.2	99.5	101.4
1.0:0.5	99.7	99.2	100.8	98.7	100.8	99.5
1.0:0.2	100.9	98.6	99.2	99.7	98.6	99.5
0.5:1.0	99.4	99.4	100.9	99.1	98.4	100.7
0.2:1.0	101.7	100.9	98.3	100.5	100.6	99.3
Mean % recovery	100.58	99.88	99.55	99.63	99.62	100.30
E_r %	−0.58	0.12	0.45	0.37	0.38	−0.30
SD	0.900	0.941	1.078	0.779	0.993	0.986
RSD (%)	0.895	0.942	1.083	0.782	0.997	0.983

Mixtures were prepared in the linearity range for NAP and DFL determination as mentioned in Table 1
I = first derivative of emission spectra, II = first derivative of constant wavelength synchronous spectra, (Δλ=165 nm), III = first derivative of constant energy synchronous spectra, E_r %: Percentage relative error, SD = standard deviation, RSD (%) = percentage relative standard deviation

All the methods were applied to the solutions thus prepared.

Analytical procedures

Determination of naproxen and diflunisal by first derivative of emission spectra

The samples were prepared as described above. The emission spectra were recorded in the range 310–460 nm with excitation at 243 nm and corrected from the blank signal for the simultaneous determination of NAP and DFL. First derivatives were calculated and the absolute values of the first derivative were measured at 327 and 434 nm for the determination of NAP and DFL, respectively. Linearity ranges are mentioned in Table 1.

Determination of naproxen and diflunisal by first derivative of constant wavelength synchronous spectra

Using this technique, two procedures were proposed. The first of which allows the determination of NAP and DFL simultaneously by recording only one synchronous spectrum when present in the linearity ranges mentioned in Table 1. The synchronous spectra were recorded by scanning both monochromators together while maintaining a constant of 165 nm difference between both wavelengths. First derivatives were calculated and the absolute values of the first derivatives were measured at 226 nm for NAP and 251 nm for DFL.

The other procedure enables NAP to be determined in the presence or absence of DFL and vice versa by recording two synchronous spectra. This was used to significantly increase the sensitivity of the method especially for the determination of NAP, as seen in Table 1. This was achieved by scanning both monochromators while maintaining a constant of 122 or 202 nm difference between both wavelengths for the determination of NAP and DFL, respectively. The absolute values of the first derivatives were then measured at 229 and 212 nm for the determination of NAP and DFL, respectively.

Determination of naproxen and diflunisal by first derivative of constant energy synchronous spectra

In this technique, the synchronous spectra were recorded in the range 240–320 nm, while maintaining a constant difference of $-8,500$ cm^{-1} between their wave-numbers. The first derivatives were calculated and the absolute values were measured at 255 and 294 to 314 nm, peak to peak, for the determination of NAP and DFL, respectively. Using this technique, both drugs were determined within the concentration ranges mentioned in Table 1.

Results and discussion

Spectral characteristics

As can be seen from Fig. 1, NAP exhibits three excitation maxima at 221, 253 and 323 nm, and one emission maximum at 343 nm (Fig. 2a). DFL presents also three excitation maxima at 205, 243 and 300 nm and its emission maximum is at 407 nm (Fig. 2a). For simultaneous determination of NAP and DFL, the excitation wavelength
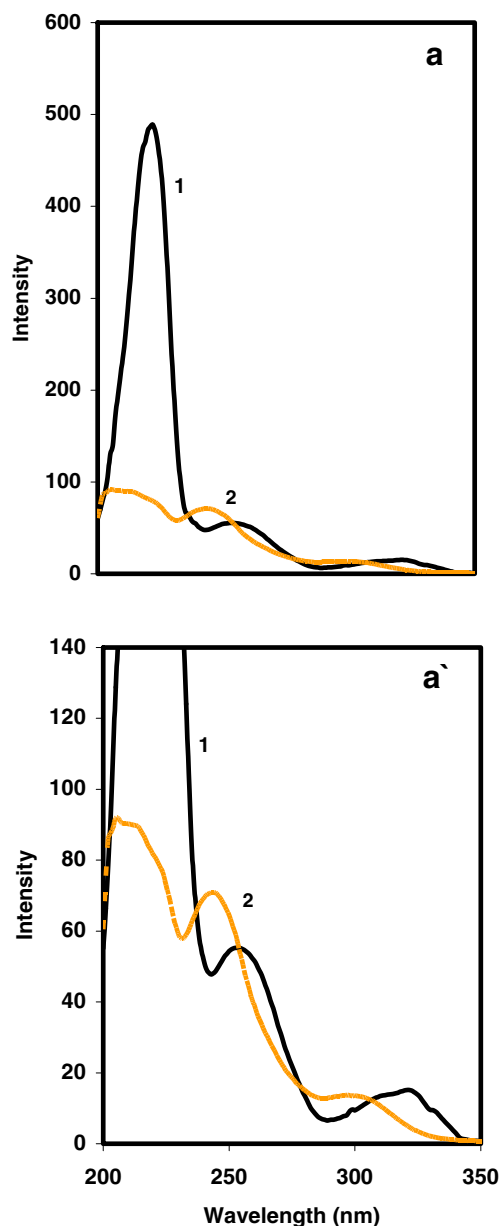

Fig. 1 Fluorescence excitation spectra of 0.1 µg ml⁻¹ NAP, 1, $\lambda_{em}=$ 343 nm and 0.1 µg ml⁻¹ DFL, 2, $\lambda_{em}=407$ nm, in mixed solvent: 40% methanol, 60% aqueous phosphate buffer pH 8.0. **a** Full spectra and **a′** the y-axis is rescaled to view the working excitation wavelengths obviously
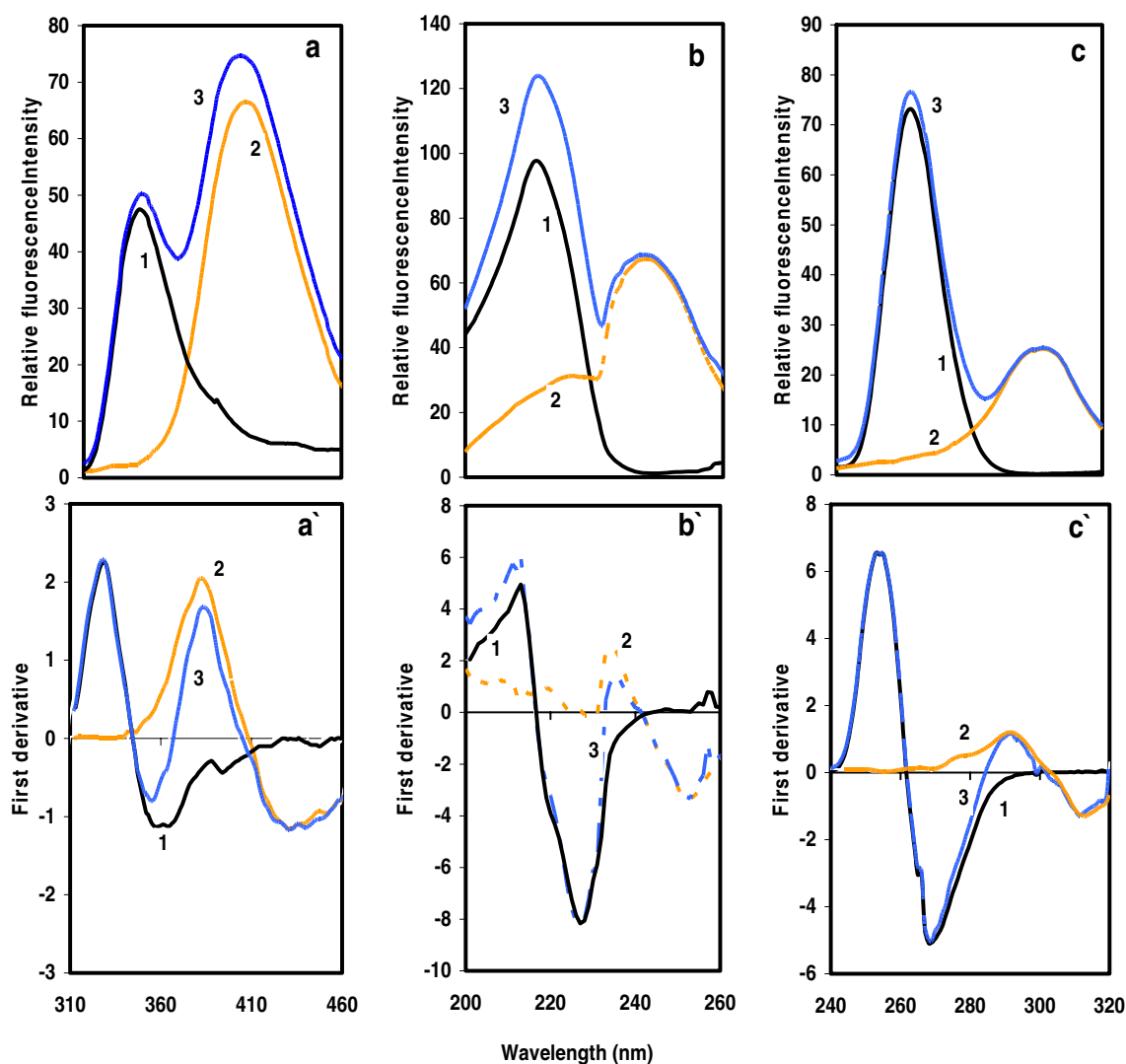
Fig. 2 Fluorescence emission spectra, λ_{ex}=243 nm, **a**, constant wavelength synchronous fluorescence spectra, $\Delta\lambda$=165 nm, **b**, constant energy synchronous fluorescence spectra, ΔE= $-8{,}800$ cm^{-1}, **c**, of 0.1 μg ml^{-1} NAP, 1, 0.1 μg ml^{-1} DFL 2, and a mixture of 0.1 μg ml^{-1} NAP and 0.1 μg ml^{-1} DFL,3, and their corresponding first-derivative spectra **a′**, **b′** and **c′**, respectively, in mixed solvent: 40% methanol, 60% aqueous phosphate buffer pH 8.0, after being corrected from the blank signals

chosen to work with was 243 nm where suitable response for both drugs was obtained. In spite of the fact that we do not obtain maximum sensitivity with this, the inner filter effect, which occurs at wavelengths close to 200 nm due to certain inorganic ions, is avoided.

Factors affecting fluorescence intensity

Chemical variables were studied to get the best measurement conditions and maximum fluorescence signals of NAP and DFL. The dielectric constant can modify the fluorescence characteristics of the compound. Thus, the effect of methanol content in the medium was investigated by preparing samples of both drugs and varying the methanol percentage between 0 and 80%. An increase in the methanol percentage of the medium results in a small increase in the fluorescence intensity of NAP while that of DFL increases significantly till methanol content of 40% after which the relative fluorescence intensity starts to decrease. Methanol content of 40% was chosen since it provided maximum sensitivity for both compounds.

The influence of pH of the aqueous phase on the fluorescence intensity was studied by adjusting the pH of the aqueous phase using o-H$_3$PO$_4$ or NaOH solutions. The relative fluorescence intensity of DFL increases with pH while that of NAP varies only very slightly. Increasing the pH results in an increase of the fluorescence intensities of both compounds till pH values of 6.0 and 8.0 where maximum response were obtained for both NAP and DFL, respectively. Aqueous phosphate buffer solutions of pH 8.0

were selected as most suitable since the fluorescence of DFL was favored.

The effect of phosphate buffer strength on the fluorescence signals of both drugs was also studied. The buffer strength has only a slight effect on the relative fluorescence of both drugs. However, maximum fluorescence intensities were obtained when using 0.02 M phosphate buffer solutions. As a result, a 0.02 M phosphate buffer solutions of pH 8.0 were found to be the best for an adequate buffering capacity. Under these conditions, the influence of both drug concentrations on fluorescence intensity was studied as mentioned in Table 1.

Resolution of binary mixtures of naproxen and diflunisal

Figure 2a illustrates that binary mixtures of NAP and DFL cannot be determined by conventional fluorescence since they show overlapping spectra. For this reason it is necessary to use other techniques. In order to discover the best instrumental conditions, scan speed and width factor were studied to ascertain the most suitable signal–noise ratio. For the three proposed methods, a scan speed of 240 nm/min and a band width of 10 nm were chosen for the analysis.

Determination of naproxen and diflunisal by first derivative of emission spectra

Figure 2a shows that there is a significant overlap between the emission spectra of both drugs so it was not possible to use them directly to determine such binary mixtures. As a result, the first derivative of emission spectra was calculated (Fig. 2a′) and the zero-crossing technique was applied to resolve the overlapping spectra. NAP was determined by measuring the first derivative signals at 327 nm, zero contribution from DFL. Similarly, DFL was determined by measuring the first derivative signals at 434 nm, zero

contribution from NAP. Although the maximum fluorescence intensities were obtained when the emission spectra were recorded at 254 and 243 nm as excitation wavelengths for NAP and DFL, respectively, 243 nm was the wavelength chosen because it was possible to determine both drugs simultaneously by scanning only one spectrum with suitable fluorescence intensity and without loss of sensitivity in respect of the maximum for each one.

Determination of naproxen and diflunisal by first derivative of constant wavelength synchronous spectra

Other fluorescence techniques used to improve the results combine conventional synchronous spectra with the derivative technique. To begin with, the method of constant wavelength synchronous spectra was tried. It was found that the maximum signal for NAP was obtained at $\Delta\lambda = 122$ nm, which corresponds to the difference between the emission and excitation maxima of NAP ($\lambda_{em} - \lambda_{ex} = 343 - 221 = 122$). The spectra obtained were used to calculate the first derivatives and the absolute values at 229 nm were related to the concentration of NAP. At $\Delta\lambda = 202$ nm, which also corresponds to the difference between the emission and excitation maxima of DFL, ($\lambda_{em} - \lambda_{ex} = 407 - 205 = 202$), best sensitivity and maximum signal were obtained for DFL by measuring the absolute values of the first derivatives at 212 nm.

However, a wavelength interval of 165 nm was used to determine both drugs by scanning only one synchronous spectrum with suitable sensitivity (Fig. 2b). Owing to the significant overlap shown in Fig. 2b, it was necessary to use the zero-crossing technique on the first derivative of these spectra to resolve the mixture (Fig. 2b′).The selected zero-crossing wavelengths were 226 and 251 nm for NAP and DFL, respectively. Instrumental conditions were the same as described above.

Table 3 Determination of NAP and DFL in pharmaceutical tablets by the proposed spectrofluorimetric methods

	% Found					
	NAP			DFL		
	I	II	III	I	II	III
Mean % recovery	99.50	100.25	99.9	99.63	99.62	100.3
E_r %	0.50	−0.25	0.1	0.37	0.38	−0.3
SD	1.108	0.997	1.088	0.779	0.993	0.986
RSD (%)	1.114	0.995	1.089	0.782	0.997	0.983

Labelled to contain 200 mg NAP and 200 mg DFL per tablet

I = first derivative of emission spectra, II = first derivative of constant wavelength synchronous spectra, ($\Delta\lambda = 165$ nm), III = first derivative of constant energy synchronous spectra, Mean % recovery = mean of five determinations, E_r % = percentage relative error, SD = standard deviation, RSD (%), percentage relative standard deviation

Table 4 ANOVA's results of NAP and DFL in pharmaceutical tablets by the proposed spectrofluorimetric methods

Source of variation	Sum of squares		Degrees of freedom		Mean squares		F-test[a]	
	NAP	DFL	NAP	DFL	NAP	DFL	NAP	DFL
Between groups	1.69	1.823	2	2	0.845	0.912	0.744	1.067
Within groups	17.035	12.822	15	15	1.136	0.855		
Total variation	18.725	14.645	17	17	–	–		

[a] The theoretical value for F at $P=0.05$ and degrees of freedom 2,15 equals 3.6823

Determination of naproxen and diflunisal by first derivative of constant energy synchronous spectra

The last method used to improve the results was constant energy synchronous spectra, where different scans of samples of NAP and DFL were recorded between 240 and 320 nm at different wave number intervals ranging from −50 to −8,888 cm^{-1}. Maximum fluorescence intensity was obtained when the wave number −8,500 cm^{-1} was used for both NAP and DFL. A wave-number of −8,500 cm^{-1} was the most suitable for resolving these mixtures by recording only one constant energy synchronous spectrum since suitable fluorescence intensity was obtained without loss of sensitivity (Fig. 2c). The first derivative (Fig. 2c′) was calculated and the zero-crossing technique was also used. In this way, both drugs were resolved with the greatest sensitivity. NAP and DFL concentrations were proportional to the signals measured at 255 and 294 to 314 nm, respectively. From Fig. 2c, it is obvious that it was necessary to use the first derivative of constant energy synchronous spectra to determine NAP and that direct zero-order spectra could not be used since DFL interferes significantly in its determination. On the other hand, NAP peaks showed nearly zero contribution at the maximum of DFL peaks (303 nm) but as the signals obtained for the latter were small, the first derivative technique was used to get better sensitivity of the analytical signals, peak to peak measurement.

Methods validation

Concentration ranges and calibration graphs

Under the above described experimental conditions, a linear relationship was observed by plotting drug concentrations against first derivative signals calculated from different fluorescence spectra (emission spectra, constant wavelength synchronous spectra and constant energy synchronous spectra) for each compound, the corresponding concentration ranges are listed in Table 1. The slopes, intercepts and correlation coefficients obtained by the linear least squares regression treatment of the results are also given. The high values of the correlation coefficients (r-values>0.999) with negligible intercepts indicate the good linearity of the calibration graphs. Standard deviation of residuals ($S_{y/x}$), of intercept (S_a) and of slope (S_b) are presented for each compound using the three methods of measurements. ($S_{y/x}$) is a measure of the extent of deviation of the found

Table 5 Determination of NAP and DFL in human serum samples by the proposed spectrofluorimetric methods

Fortification level (μg per 0.5 ml)		% Recovery						
NAP	DFL	NAP			DFL			
		I	II	III	I	II	III	
5.0	10.0	99.0	100.8	100.1	99.71	100.8	100.2	
10.0	20.0	98.3	99.1	98.2	99.25	98.5	98.8	
5.0	5.0	98.6	98.2	99.3	98.6	99.5	99.7	
10.0	40.0	99.2	98.7	100.6	99.8	98.7	98.5	
40.0	5.0	99.8	99.4	99.2	98.1	100.8	100.5	
Mean % recovery		98.97	99.24	99.48	99.09	99.66	99.54	
Er %		1.03	0.76	0.52	0.91	0.34	0.46	
SD		0.56	0.98	0.92	0.730	1.110	0.868	
RSD (%)		0.56	0.99	0.93	0.737	1.110	0.872	

I = first derivative of emission spectra, II = first derivative of constant wavelength synchronous spectra, ($\Delta\lambda=165$ nm), III = first derivative of constant energy synchronous spectra, E_r % = percentage relative error, SD = standard deviation, RSD (%) = percentage relative standard deviation

(measured) y-values from the calculated ones. The smaller the ($S_{y/x}$), the closer the points are to the linear regression line. For equal degrees of freedom, increase in the variance ratio (F-values) means increase in the mean of squares due to regression and decrease in the mean of squares due to residuals. The greater the mean of squares due to regression, the more the steepness of the regression line is. The smaller the mean of squares due to residuals, the less the scatter of the experimental points around the regression line. Consequently, regression lines with high F-values (low significance F) are much better than those with lower ones. Good regression lines show high values for both (r) and (F) values [24].

Detection limits

Limit of detection (LOD) was calculated for each compound as presented in Table 1. Detection limits were calculated according to the International Union of Pure and Applied Chemistry [25], LOD=3 s/k where s is the standard deviation of replicate determination values under the same conditions as for sample analysis in the absence of the analyte and k is the sensitivity, namely, the slope of the calibration graph.

Precision and accuracy

In order to assess the precision, as percentage relative standard deviation (RSD%), and the accuracy, as percentage relative error (E_r %), of the proposed methods, NAP and DFL were assayed in different laboratory-prepared mixtures of different ratios (Table 2). The fluorescence measurements were taken for all solutions, using the three proposed methods of analysis, and the first derivative values were calculated over the specified range. It can be seen that, for all proposed methods, the concentration of each drug in the mixture could be determined, at the selected zero-crossing points, without any interference from the other indicating good accuracy (E_r % less than 2%) and precision (RSD% less than 2%) of the proposed methods.

Analysis of naproxen and diflunisal in their pharmaceutical preparations

The proposed methods, mentioned above, were successfully applied to the determination of the two drugs in their tablets with good accuracy and precision (Table 3). In order to check the results of the applied methods, one-way ANOVA [26] test was applied to three set of five sub-samples for each drug in their commercial tablets. For this reason, F-values were computed and compared with the standard tabulated value (P=0.05). Table 4 shows that the experimental or calculated F-values did not exceed the tabulated values of F

in the analysis of variance, indicating that there was no significant difference among the methods.

Analysis of naproxen and diflunisal in serum samples

In order to demonstrate the viability of the proposed methods, serum samples previously fortified with different concentrations of NAP and DFL were treated and analyzed as described under section 2.6. The applied levels are typical to NAP and DFL levels in serum during the first 2 h after a single oral dose of 500mg of both NAP and DFL [27].

Table 5 shows that satisfactory recovery data were obtained for the samples assayed.

Conclusion

The proposed molecular fluorescence techniques (emission spectra, constant wavelength synchronous spectra and constant energy synchronous spectra) in combination with derivative spectrometry can be readily applied for the resolution of binary mixtures of NAP and DFL. These methods permit the determination of the two drugs in their laboratory-prepared mixtures, pharmaceutical preparations and fortified serum samples with high precision and accuracy. They are specific and there is no interference from any of the sample components. The method of first derivative of constant wavelength synchronous spectrofluorimetry provided the lowest detection limits for both NAP and DFL.

Overall, the proposed methods can be applied for rapid determination of the previously mentioned drug combination and have a great promise for the routine analysis of commercial formulations and quality control of such mixtures in addition to clinical or pharmacokinetic studies. The minimum sample preparation, speed of analysis and low cost are the main advantage of these methods when compared to other alternative methods reported in the literature, such as GC [9, 10], high-performance liquid chromatography [11, 12], capillary electrophoresis [13] and high-performance thin layer chromatography [14]. The proposed methods apply neither sophisticated instruments nor any separation step.

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