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Spectrofluorometric determination of naproxen in tablets

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Abstract

A rapid, selective, sensitive and simple fluorescence method was developed for the direct determination of naproxen in tablets. The tablets were triturated, dissolved in either NH_3 or NaOH solution, sonicated, filtered and then direct fluorescence emission was read at 353 nm (exciting at 271 nm). In order to validate the method the results were compared with those obtained by the USP XXIV NF 19 Pharmacopeia reference method (high performance liquid chromatography). The slope, intercept and variances which are associated with the regression coefficient calculated with bivariate least square (BLS) regression indicate that both methods are statistically comparable. The recoveries were excellent, except in tablets containing the antibiotic tetracycline. In this latter case a correction procedure is necessary.

Keywords: Spectrofluorometry; Naproxen; HPLC; Bivariate least square regression

1. Introduction

Naproxen (naprosyn) [(+)6-methoxy-α-methyl-2-naphthaleneacetic acid] is an anti-inflammatory drug related to the arylacetic or propionic acid class. It is odorless, white and crystalline, freely soluble in water at high pH ($pH \geq 8$) but practically insoluble in water at low pH. Only the D or (+) isomer exhibits dose-related anti-inflammatory, analgesic and antipyretic activity in animals, and thus only this isomer is included in naproxen formulations.

Naproxen is used in the treatment of many diseases like rheumatoid arthritis, degenerative joint disease, ankylosing spondylitis, acute gout and primary dismenorrea [1]. Like other nonsteroidal anti-inflammatory drugs, it inhibits the biosynthesis of prostaglandins [1]. Its cronic or acute administration shows toxic manifestations generally characteristic of nonsteroidal anti-inflammatory drugs, such as gastrointestinal erosion, bleeding and pathologic changes in the renal cortex, tubules and papillae.

Several analytical methods have been reported in the scientific literature for the determination of naproxen in pharmaceutical preparations and human serum, including: chemiluminiscence, [2] synchronous spectrofluorimetry, [3–6] capillary electrophoresis, [7–9] phosphorimetry, [10–13] spectrophotometry, [14–16] electrokinetic capillary chromatography, [17,18] gas chromatogra-

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phy, [19,20] high performance liquid chromatography (HPLC) [21–36] and spectrofluorimetry [37–41].

In this work, a spectrofluorometric method for the quantitation of naproxen in tablets is reported, according to a program devoted to the development of simple methods for quality control in pharmaceutical preparations [42–44]. This method is simple, rapid, selective and sensitive.

It can be noticed that many works have been published on the determination of naproxen, including conventional spectrofluorimetry [40,41]. However, the present method is more rapid and simple as compared with previous ones [40,41]. Aqueous alkaline solutions are used in order to dissolve naproxen in tablets and it is not necessary to use ethanol, methanol or other organic solvents or to adjust pH before spectrofluorometric measurements [40,41]. The result is the direct determination of naproxen in tablets in the presence of differents excipients, avoiding sample pre-treatment procedures. Only in the presence of the antiobiotic tetracycline a suitable correction must be applied.

2. Experimental

2.1. Apparatus

Fluorescence measurements were done on: (1) a Shimadzu RF-5301 PC spectrofluorometer and (2) an Aminco–Bowman luminiscence spectrometer. Both sprectrometers were equipped with a 150 W Xenon Lamp, using 1.00 cm quartz cells, slit width as 3.0 nm, excitation wavelength at 271 ± 3 nm, and emission wavelength at 353 ± 3 nm.

Absorbance measurements were done on a BECKMAN DU 640 spectrophotometer using 1.00 cm quartz cells.

HPLC procedures were carried out on a Waters 600 Pump equipped with a column Phenomenex Phenosphere 5 μ ODS C18 (250×4.6 mm). Other parameters were: mobile phase, acetonitrile: H_2O: glacial acetic acid (50:49:1); flow, 1.00 ml min^{-1}; injection volume, 20 μl; detector Waters 486 UV–visible, wavelength, 254 nm; temperature, 21 °C; time retention registered: approximately 8 min.

2.2. Reagents

Stock solutions of naproxen (400 mg l^{-1}) were prepared by dissolving naproxen (Sigma) with NH_3 0.2 M or NaOH 0.32 M and then conveniently diluting with the same solvent, in order to obtain 20 mg l^{-1} working solutions.

NaOH 0.32 M was prepared from a carbonate free solution by a suitable dilution. All solutions were prepared with distilled water stored in glass bottles.

2.3. Calibration curve

Solutions for the calibration curve were prepared by suitable dilutions of the work solution with NH_3 0.2 M or NaOH 0.32 M in each case. The linear calibration range was 0.2–2 mg l^{-1} in both cases. The fluorescence emission intensity was measured at 353 nm exciting at 271 nm.

Using the Shimadzu spectrometer, the equation for the calibration curve is $I = A + B \times C$, where I is the fluorescence intensity (in arbitrary units) and C is the concentration of naproxen in mg l^{-1}. After a weighted least-squares linear fit of the fluorescence emission data, the following values were obtained: $A = 5.295(1)$, $B = 224(1)$, $r^2 = 0.99964$ in NaOH 0.32 M and $A = 5.295(2)$, $B = 217.8(2)$, $r^2 = 0.99946$ in NH_3 0.32 M. In both cases the number of measurements (n) was ten and the measurements on each standard (p) was 3.

Another calibration curve was obtained with the same solutions using the Aminco-Bowman spectrometer. The equation for this calibration curve is $I = A + B \times C$, $A = 1.838(2)$, $B = 98.2(2)$, $r^2 = 0.9997$ in NH_3 and $A = 0.3779(1)$, $B = 67.8(1)$, $r^2 = 0.9997$ in NaOH.

2.4. Artificial and commercial samples (tablets)

Artificial ammonia and sodium hydroxide samples were prepared by addition of standard naproxen to a suitable matrix.

Pharmaceutical preparations (tablets) were obtained from the following laboratories: Roche (Aleve), Andrómaco (Naprux, NapruxGesic), Microsules Bernabó (Alidase, Papasine), Rontag (Naprontag) and processed as described below.

2.5. Procedure for pharmaceutical samples

Commercial tablets were processed as follows. An amount of triturated capsules containing 20 mg of naproxen was weighed, dissolved with NH_3 0.2 M in one case, and with NaOH 0.32 M in the other, sonicated for 20 minutes and filtered. Then final convenient dilutions with NH_3 0.2 M and NaOH 0.32 M were in each case performed in order to obtain concentrations within the linear calibration range. In all cases, it was assumed that the content of tablets corresponds to that reported by the manufacturing laboratories.

Concentrations values were selected in the central part of the calibration curve: 50, 100 and 150% of the target central value (0.90 mg l^{-1}), i.e. 0.45, 0.90 and 1.35 mg l^{-1}. This was easily accomplished taking into account the linear calibration range (0.2 and 2.00 mg l^{-1}) and the content of naproxen in tablets (80–500 mg per tablet).

2.6. Chromatographic procedure

Commercial tablets were treated as indicated in the United States Pharmacopeia using a very similar chromatographic system (see above).

3. Results and discussion

Naproxen is soluble in water only at pH > 8, and emits fluorescence at 353 nm when it is excited at 271 nm (Fig. 1), so that the quantitation of naproxen requires maintaining the appropriate pH.

The first step in the method development is the linearity of the calibration function. The electronic absorption spectrum of naproxen at pH > 8 gives an estimate about the maximum concentration at which fluorescence linearity may be expected (which corresponds to an absorbance ≤ 0.05). This value was estimated approximately as 3.0 mg ml^{-1}. In the actual fluorescence experiments the calibration plot was found to be linear in the range 0.2–2 mg l^{-1} both in NH_3 0.2 M and NaOH 0.32 M. Linear calibration functions allows to use linear regression for data treatment.

3.1. Test for the linearity of the calibration

The linearity of a calibration function is a critical analytical parameter. The correlation coefficient r^2 has often been used as a test for linearity. However, a correlation coefficient close to unity does not necessarily indicate a linear calibration function. Therefore, statistical tests for significant lack of fit to linear models were applied to the designed calibration experiments.

Standards of identical matrices in which the analyte is present at different and accurately known concentrations in the range 0.2–4 mg l^{-1} were prepared. Then, for each concentration, the emission fluorescence at 353 nm (excited at 271 nm) was measured by triplicate in random order. In our case, we found that the standard deviation of each measurement differ for each standard and therefore heterocedasticity was present. In this case the intercept A and slope B are estimated by weighted regression, which minimizes the value of $\Sigma_{ij} w_i(R_{ij} - \hat{R}_I)^2$ where R_{ij} is the experimental fluorescence intensity value for each c_{ij}, $\hat{R}_I$ is the fitted intensity, $(R_{ij} - \hat{R}_I)$ is the difference between experimental and fitted values or residuals. The

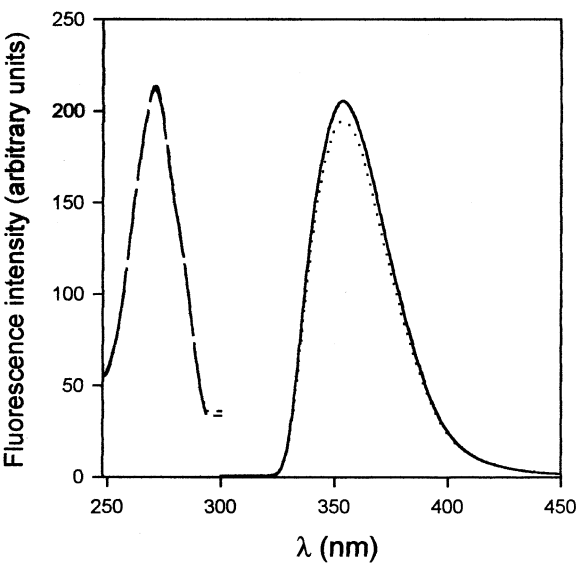

Fig. 1. Emission and excitation spectra of naproxen: (——) fluorescence emission spectrum in NaOH, (· · · ·) fluorescence emission in NH_3, (- - -) fluorescence excitation in NH_3, and in Na(OH) (the spectra are coincident).

Table 1
Calibration data for the spectrofluorometric determination of naproxen in NH_3 0.2 M and in NaOH 0.32 M[a]

Naproxen (mg l^{-1})	σ in NaOH	R.S.D.%[b]	σ in NH_3	R.S.D.%[b]
0.200	0.724	1.440	0.152	0.330
0.400	1.990	2.000	1.787	1.990
0.600	2.717	1.900	0.182	0.131
0.800	1.088	0.590	3.177	1.760
1.000	1.380	0.590	2.800	1.240
1.200	6.892	2.500	1.905	0.710
1.400	3.682	1.160	7.770	2.480
1.600	0.840	0.230	1.038	0.290
1.800	1.420	0.340	0.428	0.110
2.000	0.142	0.030	1.981	0.450

[a] Average of three determinations.
[b] R.S.D.%, relative standard deviation for each point from the regressed lines.

weight is given by $w_i = \sigma_i^{-2}$, where σ_i is the standard deviation of the fluorescence intensity at the concentration c_i.

The required weights w_i were first calculated (Table 1). A test for lack of fit based on the analysis of the residual variance was then employed. After applying the weighted least-squares method for heteroscedastic cases, the following parameters are calculated: the weighted residual sum of squares [$SS_{(W)R} = \Sigma_{ij}\, \omega_i r^2_{(w)ij}$, $MSS_{(w)R} = SS_{(w)R}/(n \times p - 2)$] where the subscript (w) indicates statistics estimated by the weighted method with $(n \times p - 2)$ degrees of freedom (n, number of standards and p, measurements made on each standard). This sum of squares can be broken down into two terms, the sum of squares due to pure error [$SS_{(w)E} = \Sigma_{ij}\, \omega_i (R_{ij} - \ddot{R}_i)^2$, with $n(p-1)$ degrees of freedom, $\ddot{R}_i = \Sigma_{ij}\, R_{ij}/p$ and $MSS_{(w)E} = SS_{(w)E}/n(p-1)$] and the sum of squares due to lack of fit [$SS_{(w)lof} = SS_{(w)R} - SS_{(w)E}$, with $(n-2)$ degrees of freedom, $MSS_{(w)lof} = SS_{(w)lof}/(n-2)$]. If the ratio $F = MSS_{(w)lof}/MSS_{(w)E}$ is found to be lower than the tabulated $F_{(n-2);\, n(p-1),}$ $P < 0.05$, the conclusion is that there is no significant lack of fit [45–47].

Both for naproxen in NaOH 0.32 M and in NH_3 0.2 M, the equation for the calibration curve was calculated by weighted least-squares linear fit and the ratio F was estimated in the ranges 0.2–4, 0.2–3 and 0.2–2 mg l^{-1}. In the first two cases, the F values were higher than the tabulated F using (18, 40) and (13, 30) degrees of freedom, respectively. In the last case (0.2–2 mg l^{-1}), for naproxen in NaOH 0.32 M, the calculated $F_{(8,20)}$ value is 2.55, lower than that tabulated (4.94, $P < 0.05$). For Naproxen in NH_3 0.2 M, the calculated $F_{(8,20)}$ is 1.62, also lower than that tabulated. We conclude that there is no statistically significant lack of fit in either NH_3 0.2 M or NaOH 0.32 M in the range 0.2–2 mg l^{-1}.

3.2. Detection limit

The detection limit, described as the lowest concentration level of the analyte that produces a response detectable above the noise level of the system (typically three times the noise level, calculated as $3\sigma_A/B$, in which σ_A is the standard deviation of the intercept of the computed fitted regression line and B is the slope), was 0.025 mg l^{-1} for NH_3 0.2 M and 0.020 mg l^{-1} for NaOH 0.32 M. However, only the error in the intercept is considered in the latter procedure. A better alternative is the equation proposed by Winefordner and Long as:

$$\mathrm{LOD} = k\left[\sigma_{\mathrm{bl}}^2 + \sigma_A^2 + \left(\frac{A}{B}\right)^2 \sigma_B^2\right]^{1/2} \quad (1)$$

where σ_{bl} is the standard deviation of a set of replicates (ten in our case) of blank sample solutions, k is a constant value usually equal to 3, σ_B is the standard deviation of the slope. Eq. (1) takes into account errors in the slope and intercept, as well as the blank noise. Applying Eq. (1),

the calculated limit of detection is 0.025 mg l^{-1} in NH_3 0.2 M and 0.020 mg l^{-1} in NaOH 0.32 M [48,49].

When the detection limit is calculated by the methods discussed above only false positive conclusions are considered. In order to consider both false positive and false negative conclusions, the detection limit was calculated as proposed by Clayton [50]:

$$\mathrm{LOD}(p, q) = \omega_0 \Delta(p, q)\frac{\sigma}{B} \quad (2)$$

where:

$$\omega_0 = \frac{1}{N} + \frac{1}{n} + \frac{\bar{x}^2}{\sum_{j=1}^{n} n_j (x_j - \bar{x})^2} \quad (3)$$

In Eqs. (2) and (3), N is the number of measurements made for each standard, n is the number of standards, $\bar{x}$ is the average of all n observations, $\Delta(p, q)$ is a tabulated value for specified p (type I error) and q (type II error) and $n-2$ degrees of freedom, $\sigma = \sqrt{\mathrm{MSS}_{(\mathrm{W})R}/(np-2)}$ (heteroscedastic case). In our case, we estimate 0.050 mg l^{-1} for NH_3 0.2 M and 0.048 mg l^{-1} for NaOH 0.32 M.

The method was fully validated applying the following tests.

3.3. Robustness of the method

In order to demonstrate the robustness of the method, changes in the alkaline pH were made. Standards solutions of naproxen at the concentration level 0.80 mg l^{-1} were prepared with the same aqueous matrix and at different alkaline pH solution in the range 9.00–14.00. The results were checked by means of an Analysis of Variance (ANOVA) test. Calculated F ratios for (3,16) degrees of freedom (1.97 for NH_3 and 3.10 for NaOH) was compared with the tabulated F value (3.24, $P < 0.05$). Taking this into account, no statistical significant difference is found in quantitation of naproxen when changes in the alkaline pH were made.

Another robustness test for the method involves proving that there are no significant differences between the quantitation of naproxen in NH_3 0.2 M or in NaOH 0.32 M. We have also checked this assertion by means of an ANOVA test: series of ten replicates of the standard with concentration 0.90 mg l^{-1} were quantitated in NH_3 0.2 M and in NaOH 0.32 M. Then an F ratio was calculated as $F_{\mathrm{calc}} = \mathrm{TLS}/\mathrm{TLE}$ (TLS, ratio between sum of squares due to the treatment with $t-1$ degrees of freedom; TLE, ratio between sum of squares due to pure error with t $(n-1)$ degrees of freedom; t, number of treatments; n, measurements made for the selected concentration). TLS represents the inter-assay error, whereas TLE is the intra-assay error. The calculated F value (0.474) is lower than that F (4.41, $P < 0.05$, (1, 18) degrees of freedom), both in NH_3 0.2 M and in NaOH 0.32 M. We can conclude that there is no statistical significant difference between both procedures.

3.4. Repeatability or intra-assay precision

This parameter represents the minimum dispersion or the maximum precision of the assay. The intra-assay precision or repeatability was studied by analyzing repeatedly, in one laboratory on the same day, three replicate aliquots of homogeneous samples, each of which was independently prepared according to the procedure described above. The results are shown in Table 2. A useful criterion for an assay method is that the intra-assay precision measured as R.S.D.% will be $\leq 2.00\%$ in the same laboratory on 1 day [51].

Table 2
Repeatability or intra-assay precision in the spectrofluorometric determination of naproxen in NH_3 0.2 M and NaOH 0.32 M

Naproxen (mg l^{-1})	R.S.D.% in NH_3[a]	R.S.D.% in NaOH[a]
0.200	0.200	0.890
0.400	1.080	1.131
0.600	0.740	0.600
0.800	1.251	0.871
1.000	0.790	0.271
1.200	0.600	1.201
1.400	1.000	0.841
1.600	0.211	0.162
1.800	0.130	0.270
2.000	0.280	0.231

[a] Average of three replicates.

Table 3
Reproducibility, aqueous NH_3 samples artificially added with naproxen measured in SHIMADZU (primary testing lab) and AMINCO spectrofluorometers[a]

Taken naproxen (mg l^{-1})	Found naproxen (mg l^{-1})				R.S.D.% interassay	
	SHIMADZU		AMINCO		NH_3	NaOH
	NH_3	NaOH	NH_3	NaOH		
0.200	0.193	0.199	0.191	0.202	0.950	0.180
0.400	0.400	0.409	0.401	0.411	0.730	0.770
0.600	0.620	0.620	0.608	0.615	1.300	0.520
0.800	0.809	0.807	0.803	0.808	0.900	1.730
1.000	1.025	1.017	1.007	1.007	1.200	1.320
1.400	1.410	1.413	1.405	1.410	0.680	0.300
1.800	1.818	1.797	1.799	1.787	0.700	0.960
2.000	2.014	2.014	1.992	1.991	0.680	1.270

[a] Average of three determinations.

3.5. Reproducibility or inter-assay precision

This parameter represents the maximum dispersion or the minimum precision of the same assay. In order to study the reproducibility, homogeneous aqueous samples were artificially added with naproxen in NH_3 and in NaOH, and were then tested by applying the presently described method in two differents laboratories, using two different spectrofluorometers (Section 2) [52].

The assay results are statistically equivalent provided the mean results are within 2% of the value obtained by the primary testing lab (in this case involving the Shimadzu Spectrofluorometer). The results are shown in Table 3. Notice that the interassay RSD% values are satisfactory ($\approx 2\%$).

3.6. Accuracy

In this work accuracy was determined in two ways: (1) analyzing the recoveries of known amounts of analyte spiked into sample matrices (Table 4), and (2) comparing test results from the presently proposed method with those from a reference method such as the one involving HPLC proposed in the United States Pharmacopeia (Section 2) [53]. For the latter test, naproxen was quantified by triplicate in all the pharmaceutical preparations. The results are shown in Table 5.

The results shown in Table 4 suggest the presently discussed method is accurate in what concerns artificially prepared samples. A statistical analysis of recoveries may be performed applying linear regression analysis of added vs. found data. Mandel and Linnig [54,55] studied the accuracy in chemical analysis using ordinary least-squares (OLS) regression to the linear relationship:

$$x_{\text{found}} = a + b \times x_{\text{taken}} \tag{4}$$

The theoretical values for the slope b and intercept a are 1 and 0, respectively. However, systematic and random errors may produce deviations of this ideal situation. After the parameters a and b are calculated from the linear fit they are conveniently compared with the ideal values 0 and 1 using the elliptic joint confidence region (EJCR) for the true slope β and intercept α, according to Mandel and Linnig:

$$n(a-\alpha)^2 + 2(\sum x_i)(a-\alpha)(b-\beta)$$

$$+ (\sum x_i)^2(b-\beta)^2 = 2s^2F^2 \tag{5}$$

where n is the number of points, s^2 the regression variance, F the critical value with 2 and $n-2$ degrees of freedom at a given confidence level, usually 95%. The boundary of the ellipse is deter-

mined by the magnitude of experimental errors and by the degrees of confidence chosen. If the point (1, 0) lies inside the ellipse drawn with the experimental points obtained with the proposed method, it can be concluded that proportional and constant biases are absent. As seen in Fig. 2A, the point (1, 0) lies inside the EJCR, so that the recoveries may be considered satisfactory.

Before comparing the results obtained for the commercial samples (Table 5) it may be noticed that no interference from the preparation excipients were observed concerning the spectrofluorimetric method (all recoveries are within the limits recommended by Pharmacopeia: 90–110% of the declared amount of naproxen), except in the case of Papasine, where the recoveries are below 90%. The latter formulation contains a mixture naproxen and the antibiotic tetracycline. The apparent interference of this latter component merits a particular discussion.

The concentration of tetracycline in Papasine is three times higher than that of naproxen (Table 5). No fluorescence emission was obtained under the conditions described above for standard solutions of tetracycline, both in NH_3 and NaOH. In order to investigate the presence of inner filter effects, electronic absorption spectra of standard solutions of tetracycline and naproxen, in NH_3 and in NaOH, prepared in the ratio present in the pharmaceutical formulation Papasine (250:80) were obtained (Fig. 3). Analysis of these spectra shows that tetracycline presents a strong absorbance at 271 nm (the excitation wavelength for naproxen) and a relatively moderate absorbance at 353 nm (the emission wavelength of naproxen). We thus assume that the reason of the interference of tetracycline in the spectrofluorometric determination of naproxen is a strong inner filter effect at the excitation wavelength and moderate inner filter effect at the emission wavelength. Both of these effects decrease the intensity of the observed fluorescence. The most important effect in this case is the inner excitation filter effect, since the absorbance of tetracycline at 271 nm is six times that of naproxen (0.490:0.088).

If a suitable correction is applied, the concentration of naproxen can be accurately calculated in the presence of tetracycline. The corrections were done by two alternative methods. The first method involves measuring the absorbances at 271 (A_{exc}) and 353 nm (A_{em}) in the pharmaceutical formulation Papasine, and the emission fluorescence intensity is corrected according to [56]:

$$I_{corr} \approx I_{obs} \times \text{antilog}\left[\frac{(A_{exc} + A_{em})}{2}\right] \quad (6)$$

After this correction was applied, the concentration of naproxen in the presence of tetracycline in the pharmaceutical formulation Papasine was recalculated and the recoveries increased up to $98 \pm 5\%$ in NH_3 and $101 \pm 3\%$ in NaOH.

The inner filter effect was also corrected by the internal standard addition. Samples of the pharmaceutical preparation Papasine diluted to a suit-

Table 4
Results for aqueous samples artificially added with naproxen[a]

Taken Naproxen (mg l^{-1})	Found Naproxen mg l^{-1} in NH_3 0.2 M[a]	REC% (R.S.D.%)	Found Naproxen mg l^{-1} in NaOH 0.32 M[a]	Recovery% (R.S.D.%)
0.200	0.198	99.0 (0.450)	0.203	101.5 (1.030)
0.400	0.400	100.0 (0.360)	0.411	102.0 (0.560)
0.600	0.607	101.0 (1.300)	0.619	103.0 (0.830)
0.800	0.809	101.1 (1.130)	0.820	102.0 (1.270)
1.000	1.014	101.0 (1.000)	1.017	101.7 (1.070)
1.400	1.415	101.0 (0.550)	1.428	102.0 (1.440)
1.800	1.819	101.0 (0.870)	1.797	99.9 (0.200)
2.000	2.015	100.8 (0.700)	2.034	101.7 (1.000)

[a] Average of three determinations.

Table 5
Comparison of the determination of naproxen in pharmaceutical preparations by spectrofluorimetry and HPLC

Preparation	Composition	Naproxen found/mg per tablet (recovery%)[a]		
		Fluorescence		HPLC
		NH_3	NaOH	
Aleve (tablet)	Naproxen (Na salt) 220 mg excipients	224 ± 1 (102.0)	219 ± 1 (99.5)	226 ± 2 (102.7)
Naprux (tablet)	Naproxen 500 mg excipients	504 ± 8 (100.8)	505 ± 4 (101.0)	510 ± 5 (102.0)
Naprux gesic	Naproxen (Na salt) 275 mg excipients	280 ± 2 (102.0)	277 ± 2 (100.7)	280 ± 3 (102.0)
Naprontag (tablet)	Naproxen 250 mg excipients	251 ± 3 (100.4)	250 ± 2 (100.0)	252 ± 5 (101.0)
Alidase (tablet)	Naproxen 250 mg starch 110 mg lactose 19 mg magnesium stearate 1 mg	253 ± 2 (101.0)	251 ± 3 (100.4)	249 ± 2 (99.6)
Papasine (tablet)	Naproxen 80 mg tetracycline 250 mg	71 ± 2 (88.7)	70 ± 2 (87.5)	79.2 ± 0.8 (99.0)

[a] Average of three determinations $\pm$ S.D. Recoveries were calculated considering that the preparations contained the amounts reported by the manufacturing laboratories.

able concentrations near the central part of the calibration curve were prepared and the fluorescence was measured at 353 nm (exciting at 271 nm). They were then spiked with four small volumes of a standard solution of naproxen and the fluorescence was measured again. The concentration of naproxen in Papasine was recalculated and the recoveries values increased up to $99 \pm 4\%$ in NH_3 and $97 \pm 4\%$ in NaOH.

Linear regression with errors in both axes was applied in order to assess the accuracy of the newly developed spectrofluorometric method by comparison with those obtained using the reference method HPLC. The test is also based on the joint confidence interval for the slope and the intercept of the regression line, which is calculated taking this uncertainties in both axes into account. The slope, intercept and variances which are associated with the regression coefficient are calculated with bivariate least square (BLS) regression [57]. The results, shown in Fig. 2B, indicate that they are statistically comparable to those provided by HPLC.

4. Conclusions

Naproxen can be directly determined in tablets in the presence of excipients without sample pretreatment procedures by using spectrofluorometric techniques. Only in the presence of tetracycline a suitable correction procedure must be applied. The apparatus and reagents used seem to be accessible even for the most simple laboratories. Tests of robustness, reproducibility, repeatability and accuracy were successfully done in order to validate the method. The recovery results are statistically comparable to those obtained by USP XXIV NF19 Pharmacopeia (2000) reference method based on high performance liquid chromatography [53]. It can be concluded that the proposed method is fully validated.

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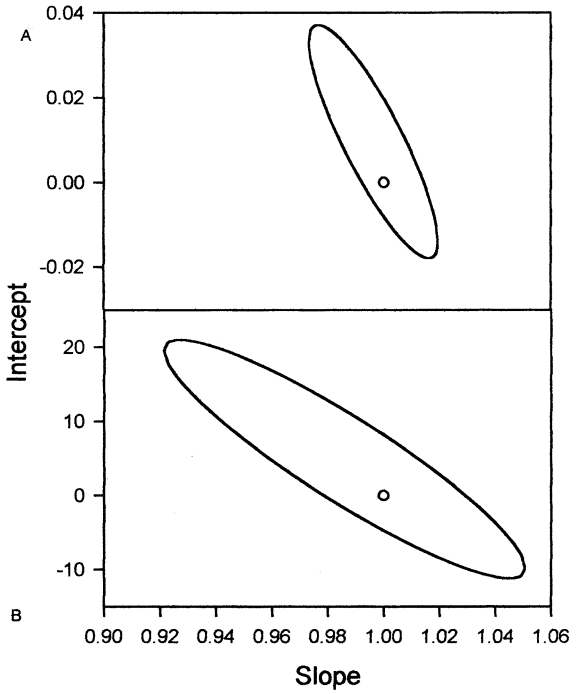

Fig. 2. (A) Joint elliptical region corresponding to the slope and intercept applying OLS for the study of the accuracy of the proposed analytical method based on the use of recovery assay on samples, (B) The same test applying BLS, (see text) for the study of the accuracy of the proposed analytical method based on the comparison with HPLC. Both in A and B, the circle marks the theoretical (1,0) point.

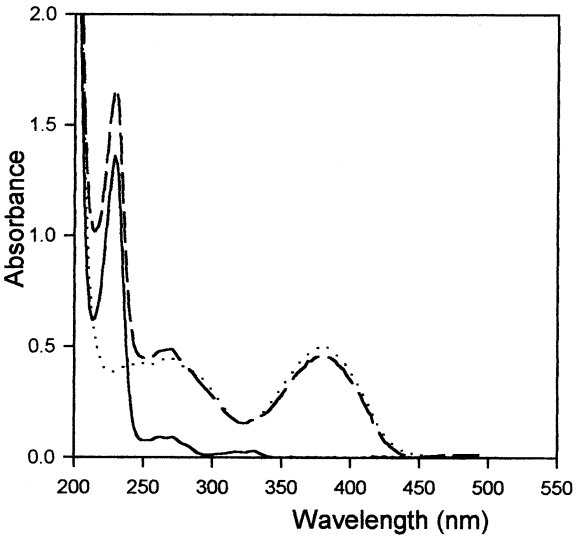

Fig. 3. Absorbance spectra of: (——) naproxen (4.1 mg l^{-1}), (· · · ·) tetracycline (12.8 mg l^{-1}) and (- - -) a diluted solution of the pharmaceutical formulation Papasine (containing a mixture of naproxen 4.1 mg l^{-1} and tetracycline 12.8 mg l^{-1}).

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