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Fluorimetric determination of procaine in pharmaceutical preparations based on its reaction with fluorescamine

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Abstract

A simple spectrofluorimetric analysis of a local anaesthetic named procaine using a specific labelling reagent for primary amino groups, has been developed. Because procaine shows very weak native fluorescence, the technique of spectrofluorimetry has been very much limited for its determination. A detail study of the variables affecting the derivatisation reaction (pH, fluorescamine (FC) concentration, temperature, reaction time), have minuciously been studied. The minimum detectable quantity is estimated as 7.7 ng ml^{-1}, with a relative standard deviation of 2.16% (ten determinations) for a procaine concentration of 100 ng ml^{-1}. The present method can be employed for the analysis of procaine by direct fluorescence measurements, without the interference from other compounds. The applicability of the present methodology have been demonstrated analysing three pharmaceutical preparations containing the analyte with satisfactory results.

Keywords: Procaine; Pharmaceutical Analysis; Spectrofluorimetry; Fluorescamine

1. Introduction

The control of drug quality is a branch of analytical chemistry that has a wide impact on public health, so the development of reliable, quick and accurate methods for determination of active ingredients is welcomed.

Procaine, also named as novocaine or neocaine (2-diethylaminoethyl *p*-aminobenzoate hydrochloride) is used as a local anaesthetic [1,2] in a wide variety of pharmaceutical preparations as an injection with antibiotic properties. It is less toxic than cocaine, but because of its poor penetration of intact mucous membranes is useless for surface application.

Several methods for the determination of procaine and its salts in pharmaceutical preparations are available in the literature [3–5]. Most pharmacopoeias [6–8] have recommended the determination of the pure substance by a tedious and rather

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inaccurate method based on a diazotisation of the primary amino group by titration with 0.1 N sodium nitrite but the determination of the active ingredient in pharmaceutical preparations requires a preliminary extraction followed by ultraviolet spectrophotometry or densitometry.

There are other analytical methods that involve separation techniques such as HPLC [9–11] or GLC [12] with detection carried out by direct or indirect spectrophotometry [13–16], infrared spectrometry [17], electron capture gas chromatography [18], polarimetry [19] and selective electrodes [20,21].

Methods involving fluorescence spectrometry have been widely applied to analytical problems which require highly sensitive detection [22]. The sensitivity and selectivity of fluorimetric methods have encouraged their wide spread use in the analysis of numerous organic compounds, and many methods for generating strongly fluorescent derivatives, have been devised. Due to the fact that procaine shows very weak native fluorescence very few luminescence methods have been found in the literature. García Sánchez and co-workers [23] published the first fluorimetric determination of procaine based on the enhancement effect of inclusion complex formed with cyclodextrins and, previously, a phosphorescence methodology was carried out for the analyte [24].

Several routes for the production of fluorescent derivatives of primary amines, are well-established. Among the most common routes are the use of derivatisating agents such as fluorescamine (FC), which was introduced by Undefriend et al. [25], which has proved usefullness in numerous analytical applications for more than 30 years [26–30].

The aim of this work was to develop a sensitive and simple spectrofluorimetric method for the analysis of the weak fluorescence analyte procaine, of pharmaceutical interest, using an specific derivatisation reaction with the well-known labelling reagent FC that constitute an interesting alternative to other analytical methods previously proposed for the analysis of this compound in pharmaceutical preparations, without any further separation required.

2. Experimental

2.1. Instrumentation

All recordings of uncorrected luminescence spectra and measurements of fluorescence intensities were carried out with an Aminco Bowman series 2 luminescence spectrometer equipped with a 7 W pulsed xenon lamp. Measurements required a personal computer with 4 MB RAM memory, DOS 6.0, OS/2 version 2.0 and a GPIB(IEEE-488) interface card for computer instrument communication.

The cell compartment was thermostatically controlled at 25.0 ± 0.5°C with a water bath circulator (S-382 Frigiterm).

A Crison Digit-501 pH meter was used for all pH measurements.

2.2. Reagents and solutions

A 100 mg sample of FC (Sigma, Sigma-Aldrich Química, Madrid, Spain) was dissolved in 100 ml of anhydrous reagent grade acetone and 'aged' for 24 h, by standing at room temperature, prior to use.

A total of 2.5 mg of procaine (Sigma) was dissolved in 1.25 ml of ethanol and diluted to 100 ml with water. The solution was stable for about 1 month.

Molar solutions of sodium citrate, monobasic potassium phosphate, dibasic potassium phosphate, sodium hydroxide, boric acid, sodium tetraborate, acetic acid and sodium acetate were prepared in the pH ranges, using the pH meter.

All solvents used were of analytical reagent grade (Merck, Merck Farma y Química S.A., Barcelona, Spain) and only demineralised water was used in this work.

2.3. General procedure

Aliquots of the procaine working solution ranging from 0 to 250 ng ml^{-1}, 3 ml of pH 4.0 citrate/dibasic phosphate buffer solution and 400 μl of the FC solution (1000 μg ml^{-1}) were transferred to 10 ml volumetric flasks and the contents were mixed again and diluted to the mark with water.

After standing for 5 min, the solutions were scanned on the spectrofluorimeter. The relative fluorescence intensity (RFI) was measured at 494 nm with excitation at 402 nm, against a reagent blank.

2.4. Sample preparation

Three different commercial products were analysed: Neocolan (Laboratorio Seid, S.A., Barcelona, Spain) with a nominal content of 424.27 mg g^{-1} of procaine and also containing: dicyclomine (16.97 mg g^{-1}), (methoxi-4-phenyl-3 (dimethoxi-2,4-phenyl)-1-oxo-propene 2 (424.27 mg g^{-1}) and excipients; Oftalmol (Laboratorio Reig Jofré, S.A., Barcelona, Spain) with a nominal content of 1.36 mg ml^{-1} of procaine and also containing: mercuric cyanide (0.03 mg ml^{-1}), boric acid (18.00 mg ml^{-1}), picric acid (0.02 mg ml^{-1}), naphazoline nitrate (0.001 mg ml^{-1}) and excipients; Sulmetin (Laboratorios Semar, S.A., Barcelona, Spain) with a nominal content of 2.0 mg ml^{-1} of procaine and containing: magnesium sulphate (120 mg ml^{-1}) and bidistilled water. A portion of the three products was dissolved in 1.25 ml of ethanol. Then, they were diluted with double distilled water to obtain a final procaine concentration of 25 μg ml^{-1} Aliquots of this solution were treated as indicated in Section 2.3.

3. Results and discussion

3.1. Fluorescence properties

An aqueous solution containing pure procaine was measured, in order to determine its excitation and emission wavelengths. This compound shows a weak native fluorescence at 345 nm, when excited at 290 nm so it was taken into account the possibility of obtaining an intense fluorescence derivative using a derivatisation reaction. Because of the amine character of procaine, the labelling reagent FC was selected.

It can be seen in Fig. 1 that, as expected, an intense fluorescence emission was obtained after the labelling reagent was carried out, with a markedly bathochromic shift of both excitation and emission spectra with respect to the normal fluorescence spectra. The final wavelengths obtained are 402 and 494 nm for the excitation and emission, respectively, so the sensitivity and selectivity of the analytical determination is greatly increased.

3.2. Experimental conditions for the derivatisation reaction

Different experimental conditions, specially pH and FC concentration, should be carefully selected because they could greatly affect the quantum efficiency of the labelling reaction.

Since it was indicated by Silva and Strojny [26] compounds possessing a the primary aromatic amino group react with FC optimally at acidic pH (between 3 and 4), so attempts were made to improve pH control in the labelling reaction step. It has been carried out several assays of solutions containing 100 ng ml^{-1} of procaine and 3 ml of different buffer solutions that covered a wide pH range (2–10). Fig. 2 illustrates the fluorescence intensity of the labelled analyte versus pH. As it can be observed in this figure, when the pH of the final solution is adjusted in the range 3.5–4.5, maximum fluorescence intensity was achieved because greatest differences between the analyte and that of a blank solution is obtained. Also, it can be deduced from this figure that a diminution of the fluorescence intensity is shown from neutral to basic pHs, which are FC-labelling conditions characteristic of aliphatic amino group-containing compounds.

As a result of this experience, it is necessary to maintain a pH 4.0 as optimum to obtain the best quantum efficiency of the labelling reaction. Several buffers of different composition could be used but the best results were obtained with a citrate/dibasic phosphate buffer solution 0.15 M of pH 4.0.

The effect of FC concentration on fluorophore formation was observed by measuring the fluorescence intensity at different FC/procaine concentration ratios, while all other experimental conditions were kept constant at the optimum values. Fig. 3 shows that maximum response was obtained when the FC/analyte concentration ratio

was within 150–500-fold concentration excess; in subsequent work, a ratio of 150:1 was employed as the minimum for the determination of procaine throughout the rest of the experimental work.

The possibility of enhancing the sensitivity of the fluorophore by using different organic solvents, was also investigated. No significant changes were observed with medium dielectric constant solvents such as methanol, propanol, ethanol and water so, consequently, the aqueous buffer medium was chosen for the experimental work.

The order of mixing of the analyte, buffer, water and FC to obtain the labelled compound, was examined. No appreciative changes were observed, so the sequence analyte, buffer, FC and water was chosen for the present study.

The emission spectrum of procaine formed between FC were measured at temperatures of 15 and 40°C. From the results obtained, no changes were observed between the fluorescence maxima; thus, in order to simplify the methodology, a constant temperature of 25°C was maintained, using a water-bath circulatory throughout the sample compartment. The samples prepared under these conditions, remained stable for, at least, 3 days after their preparation.

Also, the fluorescence intensity of the derivatised compound was measured at 5-min intervals obtaining that a reaction time of 5 min is necessary to complete the labelling reaction and, after that time, the fluorescence measurements could be carried out during 2 h.

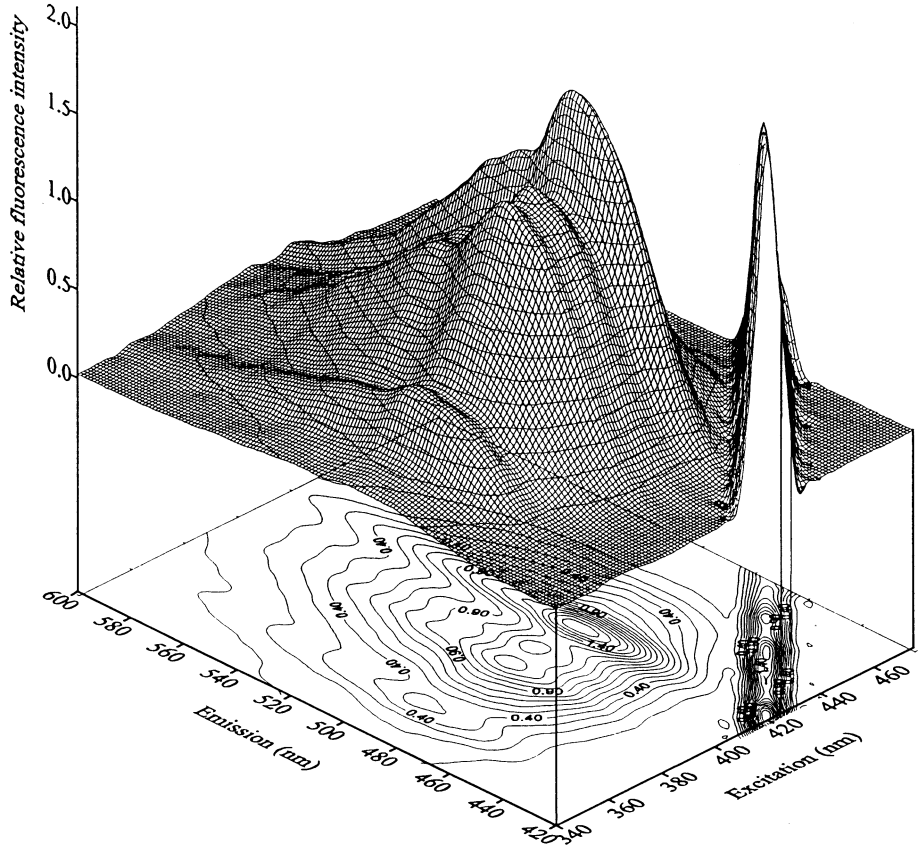

Fig. 1. Projected 3D and 2D spectra of procaine derivatised with FC. [Procaine] = 100 ng ml^{-1}, pH 4.0, [FC] = 40 μg ml^{-1}. $Slits_{exc/em}$ 8/8 nm, scanning speed 2 nm s^{-1}, and detector sensitivity 600 V.

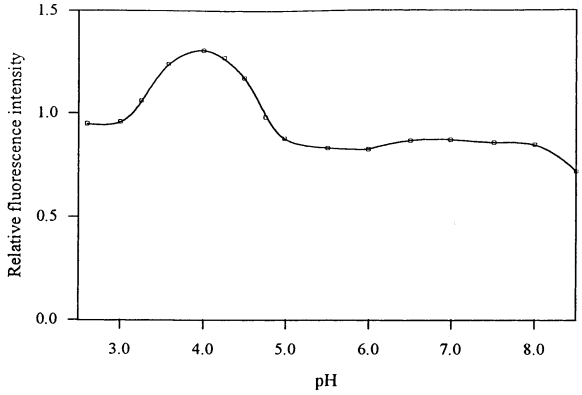

Fig. 2. Influence of pH on the relative fluorescence intensity of procaine. [Procaine] = 100 ng ml^{-1}, [FC] = 40 μg ml^{-1}. $\lambda_{exc/em}$ 402/494 nm, $slits_{exc/em}$ 8/8 nm and detector sensitivity 600 V.

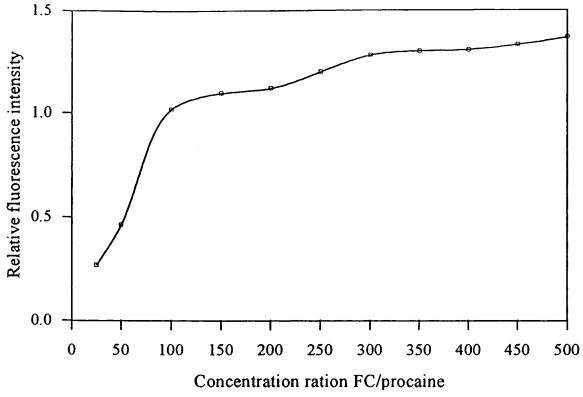

Fig. 3. Influence of the FC/procaine molar relation on the relative fluorescence intensity. [Procaine] = 100 ng ml^{-1} and pH 4.0. Instrumental conditions as in Fig. 2.

3.3. Calibration, sensitivity and precision

From the results obtained in the experimental section, the fluorescence intensity of the procaine derivatised with FC was proportional to the concentration of procaine over the range 0–250 ng ml^{-1} and the total concentration of procaine can be calculated using the corresponding correlation equation with a correlation coefficient (r) = 0.999 for $n = 7$ with a detection limit of 7.7 ng ml^{-1} [31].

The precision of the proposed method was studied by determination of the drug in seven replicates samples, individually labelled with FC at two concentration levels (100 and 250 ng ml^{-1}) obtaining relative standard deviations of 2.16 and 1.02%, respectively.

Table 1
Application of the proposed method to pharmaceutical preparations

Sample (laboratory)	Content	
	Nominal	Found[a]
Neocolan (Seid)	424.27 mg g^{-1}	392.03 ± 8.14 mg g^{-1}
Oftalmol (Reig Jofré)	1.36 mg ml^{-1}	1.23 ± 0.02 mg ml^{-1}
Sulmetin (Semar)	2.00 mg ml^{-1}	1.81 ± 0.01 mg ml^{-1}

[a] Avarage values ± standard deviation of seven determinations.

3.4. Analytical application

The proposed method was applied satisfactorily to the determination of procaine in three different pharmaceutical preparations named *Neocolan*, *Oftalmol* and *Sulmetin* with different nominal contents of procaine and other analytes, including excipients.

The results obtained for the analysis of the drug procaine in the pharmaceutical preparations using a direct spectrofluorimetric method are given in Table 1.

A paired t-test was used to compare the nominal content of procaine in the different commercial formulations with the values obtained, with $P = 0.42$ not being significant.

4. Conclusions

From the results obtained, FC is a useful reagent for the fluorimetric determination of compounds with a primary amine group which are non-fluorescent or weakly fluorescent being a simple and useful tool for analytical chemist to establish good analytical methods in complex mixtures in many fields, especially in pharmaceutical and environmental fields.

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