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Rapid methods for determination of fluoxetine in pharmaceutical formulations

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Abstract

Two different analytical methods for the quality control of fluoxetine in commercial formulations have been developed and compared: a spectrofluorimetric method and a capillary zone electrophoretic (CZE) method. The fluorescence emission values were measured at $\lambda = 293$ nm when exciting at $\lambda = 230$ nm. The CZE method used an uncoated fused-silica capillary and pH 2.5 phosphate buffer as the background electrolyte. The extraction of fluoxetine from the capsules consisted of a simple one-step dissolution with methanol/water, filtration and dilution. Both methods gave satisfactory results in terms of precision; the best results were obtained for the electrophoretic method, with RSD% values always lower than 2.0%. The accuracy was assessed by means of recovery studies, which gave very good results, between 97.5 and 102.6%. Furthermore, both methods also have the advantage of being very rapid.

Keywords: Fluoxetine; Spectrofluorimetry; Capillary electrophoresis; Pharmaceutical formulations

1. Introduction

In the last years, several new antidepressants have been introduced in the drug market, especially selective serotonin reuptake inhibitor (SSRI) antidepressants. In fact, several clinical studies emphasize that the SSRIs have good efficacy, high tolerability, and a very low risk of overdose lethality. Furthermore, the anticholinergic, antihystamine and antiadrenergic adverse effects, which are common during therapy with traditional tricyclic antidepressants (TCAs), are also reduced [1–3].

Among SSRIs, Fluoxetine (D,L-*N*-methyl-3-phenyl-3-[(α,α,α-trifluoro-*p*-tolyl)oxy]propylamine) (Fig. 1a) is one of the most widely used in therapy: its efficacy is similar to that of traditional TCAs, but at much lower doses (10–20 mg day^{-1}) [4]. Therefore, it is often the drug of choice in the treatment of severe depressive disorders [5–7].

Higher doses (about 60 mg day^{-1}) of fluoxetine seem to be suitable for the treatment of bulimia nervosa and obsessive-compulsive disorders, while

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lower doses (5–10 mg day^{-1}) have been successfully used to treat panic fits [2,5,6].

Fluoxetine hydrochloride is most widely marketed as Prozac® (Eli Lilly) and it is commercially available in several countries as capsules, tablets or solution. In fact, generic and galenic formulations of fluoxetine hydrochloride have been marketed in 30 countries by at least 48 manufacturers [8] and countless pharmacies. Many different synthetic routes have been described to manufacture fluoxetine hydrochloride [9]; furthermore, galenic preparations containing fluoxetine often contain unsuitable excipients (e.g. lactose) which can complex or react with the drug, thus inactivating it [8,10].

Several papers are available in the literature on the analysis of fluoxetine in biological fluids by means of several different techniques such as gas chromatography [11,12] and HPLC with UV [13–17], fluorimetric [18–21] or mass spectrometry [22] detection. Recently, high-sensitivity capillary electrophoresis has been used for the stereoselective separation of fluoxetine enantiomers in plasma and serum [23]. Some papers on the quality control of pharmaceutical formulations containing fluoxetine also exist; the determinations are usually based on spectrophotometry [24,25] or spectrofluorimetry [26] after derivatization, electrochemical techniques [27], gas [28] or liquid [29] chromatography and capillary electrophoresis techniques (capillary zone elctrophoresis [30] or isotacophoresis [31]). Official pharmacopoeias, such as the British Pharmacopoeia [32] and the United States Pharmacopeia [33] report the use of liquid chromatography with UV detection for the determination of fluoxetine in capsules.

In the last few years we have developed some analytical methods for the quality control of Prozac® capsules [34], based on spectrophotometry and liquid chromatography. Two alternative analytical methods based on molecular emission spectrofluorimetry and capillary zone electrophoresis are described herein. Both methods resulted to be suitable for the rapid and reliable determination of fluoxetine in Prozac® and galenic capsules.

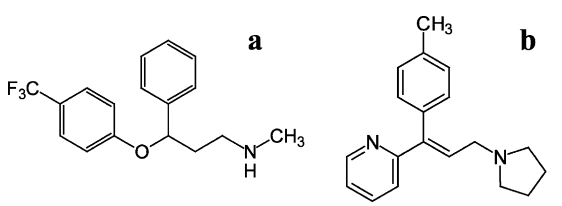

Fig. 1. Chemical structures of (a) fluoxetine and (b) triprolidine (I.S. for the electrophoretic method).

2. Experimental

2.1. Chemicals

Fluoxetine hydrochloride (99% purity) was kindly provided by Eli Lilly Italia S.p.A. (Sesto Fiorentino, Florence, Italy). Methanol was spectrometric grade from Carlo Erba (Milan, Italy). Triprolidine used as the Internal Standard (I.S.) for the capillary electrophoretic method was kindly provided by Sigma Pharmaceuticals (St. Louis, MO, USA). Ultrapure water (18.2 mΩ cm) was obtained by means of a MilliQ apparatus by Millipore (Milford, MA).

Each Prozac® capsule (Eli Lilly S.p.A.) contains a declared amount of 22.4 mg of fluoxetine hydrochloride, which corresponds to 20 mg of fluoxetine free base, and starch (205.64 mg) and dimethylpolysiloxane (2 mg) as excipients.

Each galenic capsule (prepared in an Italian pharmacy) contains a declared amount of 11.2 mg of fluoxetine hydrochloride, which corresponds to 10 mg of fluoxetine free base.

2.2. Apparatus and electrophoretic conditions

For the spectrofluorimetric assays a Jasco (Tokyo, Japan) LS-3 spectrofluorimeter and a ISA Jobin Yvon–Spex (Longjumeau Cedex, France) FluoroMax-2 spectrofluorimeter were used. Fluorescence emission intensity was measured at $\lambda = 293$ nm while exciting at $\lambda = 230$ nm.

For the capillary electrophoretic assays a Bio-Rad (Hercules, CA) BioFocus 2000 apparatus was used. The detector was operated at 205 nm. The electrophoretic runs were carried out in a 50 μm I.D. untreated fused-silica capillary (Supelco, Bellefonte, PA), with a total length of 42 cm and

an effective length of 37.2 cm. The background electrolyte was a pH 2.5, 20 mM phosphate buffer. A constant voltage of 25 kV was applied to obtain the separation. Typical current levels were less than 30 μA. Injection was carried out by pressure at the anodic end of the capillary: 10 p.s.i. for 10 s (1 p.s.i. = 6.9×10^{-3} MPa).

Before use, the new capillary was purged with deionized water for 5 min, then washed with 1.0 N sodium hydroxide for 10 min, with 0.1 N sodium hydroxide for 10 min, with water for 30 min, and finally with CZE buffer for 10 min. After each run the capillary was rinsed with buffer (1 min). For storage overnight, the capillary was washed with water and 1 M sodium hydroxide, and then again with water (rinsing time was 5 min, each). All washings were carried out at a pressure of 5 bar.

2.3. Solutions

- Fluoxetine stock solution (1000 μg ml^{-1}) was prepared by dissolving 22.4 mg of fluoxetine hydrochloride in 20 ml of a methanol–water (1:1, v/v) mixture; standard solutions were obtained by diluting the stock solution with the same mixture (fluorimetric method), or with a pH 2.5, 2.0 mM phosphate buffer (electrophoretic method).
- Triprolidine (I.S.) stock solution (1000 μg ml^{-1}) was prepared by dissolving 11.3 mg of triprolidine hydrochloride in 10 ml of methanol; standard solutions were obtained by diluting the stock solution with a pH 2.5, 2.0 mM phosphate buffer.
- Prozac® and galenic formulation stock solutions containing fluoxetine (nominal concentration: 1 mg ml^{-1}) were prepared by removing, as completely as possible, the contents of 20 capsules and mixing. An accurately weighed portion of the powder, equivalent to 20 mg of fluoxetine free base, was transferred into a test tube with 20 ml of methanol–water (1:1, v/v) mixture and, after agitation, was stored for 5 min at 4 °C. It was successively centrifuged for 15 min at 3000 rpm. Finally, the surnatant was filtered through a Whatman 540 filter paper. Working solutions of the pharmaceutical formulations were prepared exactly as the standard solutions (i.e. diluting with water/methanol for the spectrofluorimetric method and with a pH 2.5, 2.0 mM phosphate buffer for the CZE method). The test for uniformity of content of fluoxetine in capsules of Prozac® and galenic formulations was also carried out according to the directions of the British Pharmacopoeia 2000 [35].

The resulting solutions were preserved in tight, light-resistant containers, and were stable for at least 1 month at 4 °C.

The BGE for the electrophoretic method was prepared by mixing a suitable volume of concentrated phosphoric acid (85%, w/w) with water to obtain a phosphate concentration equal to 20 mM, then bringing the solution to pH 2.5 with 2 M NaOH.

2.4. Analytical procedures

2.4.1. Spectrofluorimetric method

A ten-point calibration curve was set up by plotting fluorescence emisson values against fluoxetine standard solution concentrations, in the 0.25–5.00 μg ml^{-1} range.

2.4.2. CZE method

The analyses were performed injecting fluoxetine standard solutions in the 5–50 μg ml^{-1} range. A ten-point calibration curve was set up by plotting the values of fluoxetine/I.S. peak area ratios against fluoxetine concentrations.

For both methods, six replicates of each concentration were analyzed to obtain the calibration curves.

2.4.3. Validation of the Analytical Methods

Stock solutions of the formulations were diluted, then analyzed. The percentage of drug found of the declared value was calculated interpolating on the calibration curve the results thus obtained. Solutions extracted from formulations at nominal concentrations of 0.25, 1.00 and 5.00 μg ml^{-1} were analyzed with the spectrofluorimetric method, while solutions at nominal concentrations of 5, 20 and 50 μg ml^{-1} were analyzed by

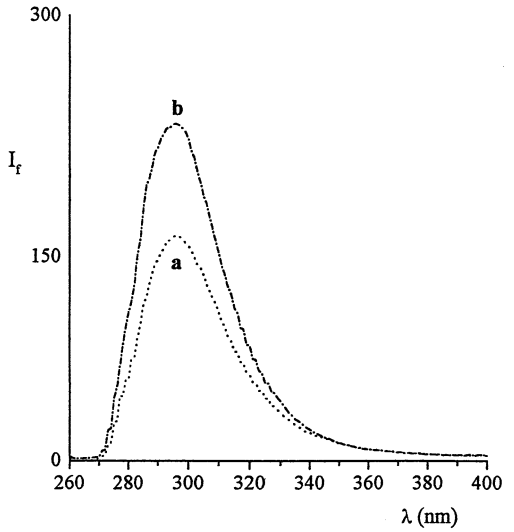

Fig. 2. Emission spectra of methanolic solutions obtained from the extraction of (a) Prozac® and (b) galenic formulation capsules (nominal concentration of both solutions: 5 μg ml^{-1}).

means of the CZE method. These assays were repeated 6 times in the same day to obtain repeatability values and 6 times over 6 different days to obtain intermediate precision values.

In order to verify the accuracy of the method, recovery assays were carried out. Known amounts of fluoxetine powder were added to the formulation powders, then extracted, diluted and analyzed. The final nominal concentrations of fluoxetine were 1.25, 2.00 and 5.00 μg ml^{-1} for the spectrofluorimetric method (i.e. additions of 0.25, 1.00 and 4.00 μg ml^{-1} to a formulation solution with a nominal fluoxetine concentration of 1.00 μg ml^{-1}) and 25, 30 and 40 μg ml^{-1} for the CZE method (additions of 5, 10 and 20 μg ml^{-1} of fluoxetine to a formulation solution with a nominal fluoxetine concentration of 20 μg ml^{-1}). These assays were repeated 6 times over 6 different days to obtain intermediate precision data.

3. Results and discussion

3.1. Spectrofluorimetric method

Fluoxetine is a fluorescent molecule; it is thus possible to determine its concentration by means of spectrofluorimetry without any previous derivatization. Preliminary studies showed that fluoxetine in alcoholic solutions has a fluorescence emission spectrum with a maximum at $\lambda = 293$ nm when exciting at $\lambda = 230$ nm. These wavelengths were used for all spectrofluorimetric assays to obtain the highest possible sensitivity.

Good linearity was obtained in the 0.25–5.00 μg ml^{-1} fluoxetine concentration range. The linearity equation obtained by means of the least-square method was $y = 1.12 + 30.96x$ ($r_c = 0.998$), where x is the fluoxetine concentration, expressed as μg ml^{-1}, and y is the emission intensity, expressed as arbitrary units. The LOQ and LOD were calculated according to USP XXIV [36] guidelines, and were 200 and 70 ng ml^{-1}, respectively. Precision assays were carried out on standard solutions at three levels (0.25, 1.00 and 5.00 μg ml^{-1}), and the results were satisfactory: the RSD% values obtained ranged from 1.5 to 2.2% for repeatablity (intraday precision) and from 2.1 to 3.9% for intermediate (interday) precision.

3.2. Application to pharmaceutical formulations

The spectra of solutions obtained from the extraction and dilution of capsules are morphologically identical to those of standard solutions (Fig. 2a, Prozac®, and Fig. 2b, galenic formulation). The mean amount of fluoxetine found of the declared value was very close to 100% for Prozac®, however, it was much higher for the galenic formulation; these values, as well as those of precision, assessed as RSD% values, are reported in Table 1.

Accuracy was assessed by means of recovery assays at three different concentrations (0.25, 1.00 and 4.00 μg ml^{-1}); the recovery results, which were very close to 100% in all cases, are reported in Table 2.

Table 1
Fluorimetric determination of fluoxetine in formulations

Concentration (μg ml^{-1})	Formulation	Repeatability[a]		Intermediate precision[a]	
		% Found/declared	RSD%	% Found/declared	RSD%
0.25	Prozac®	98.1	3.5	98.8	3.8
	Galenic	138.7	3.9	140.4	4.2
1.00	Prozac®	98.9	2.7	99.9	2.9
	Galenic	141.1	3.3	141.8	3.6
5.00	Prozac®	99.4	1.8	100.3	1.9
	Galenic	140.8	2.9	142.2	3.2

[a] $n = 6$.

3.3. Capillary electrophoresis method

The starting point of this investigation was a study recently published on the enantiomeric separation of fluoxetine and norfluoxetine in human plasma and serum [23]; however, several experimental conditions were changed in order to obtain a more simple and rapid procedure. A different capillary was used; the high-sensitivity cell and the cyclodextrins in the BGE were not necessary; the detection wavelength was changed from 195 to 205 nm to obtain a less noisy baseline. The I.S. was also changed: in this paper triprolidine (Fig. 1b) was used instead of propranolol, because triprolidine is more structurally similar to fluoxetine.

Under the leading conditions reported in Section 2, fluoxetine is detected as a neat electrophoretic peak at a migration time (t_m) of 2.4 min, while the I.S. is detected at $t_m = 1.8$ min.

A calibration curve was set up on standard solutions in the 5–50 μg ml^{-1} concentration range. The least-square regression equation was $y = -0.042 + 0.169x$ ($r_c = 0.9998$), where x is the fluoxetine concentration, expressed as μg ml^{-1}, and y is the fluoxetine/I.S. peak area ratio, a dimensionless number. Good linearity was found also in a broader concentration range (0.25–50.00 μg ml^{-1}), however, the narrower calibration curve was used for all assays. In fact, this calibration was more reliable for the determination of high amounts of fluoxetine often found in the formulations. The LOQ was 0.25 μg ml^{-1} and the LOD was 0.1 μg ml^{-1}. Both parameters were calculated according to USP XXIV guidelines [36]. Precision assays were carried out on standard solutions at three levels (5, 20 and 50 μg ml^{-1}), and the results were very good: RSD% values ranged from 0.9 to 1.2% for repeatability and from 1.1 to 1.7% for intermediate precision.

3.4. Application to pharmaceutical formulations

The fluoxetine extract in water–methanol was diluted with a pH 2.5, 2.0 mM phosphate buffer in order to obtain solutions at different nominal concentrations. The electropherograms of solutions obtained from the extraction and dilution of Prozac® and galenic capsules are reported in Fig. 3a (Prozac®) and b (galenic). As it is shown in the graphics, the analyte is detected as a neat and symmetrical electrophoretic peak in both cases. The mean amount found of the declared value and the mean precision, assessed as RSD% values, are detailed in Table 3.

Table 2
Accuracy of the fluorimetric assay

Fluoxetine added (μg ml^{-1})	Formulation	% Recovery*	RSD%*
0.25	Prozac®	100.8	3.8
	Galenic	98.3	4.1
1.00	Prozac®	100.6	2.6
	Galenic	98.1	2.7
4.00	Prozac®	98.8	1.4
	Galenic	97.5	1.7

* $n = 6$; intermediate precision.

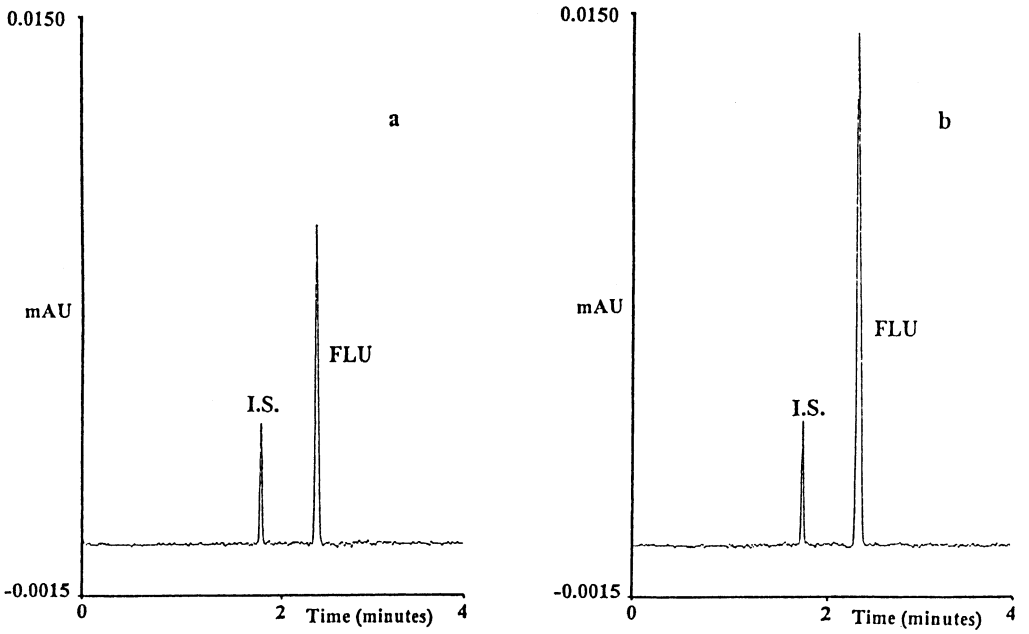

Fig. 3. Electropherograms obtained from the extraction of (a) Prozac® and (b) galenic formulation capsules (nominal concentration of both solutions: 20 μg ml^{-1}).

Table 3
Capillary electrophoresis determination of fluoxetine in formulations

Concentration (μg ml^{-1})	Formulation	Repeatability[a]		Intermediate precision[a]	
		% Found/declared	RSD%	% Found/declared	RSD%
5	Prozac®	101.2	0.9	101.5	1.9
	Galenic	142.7	1.5	144.6	1.8
20	Prozac®	101.1	1.5	102.6	1.3
	Galenic	143.1	0.5	144.0	0.9
50	Prozac®	100.5	0.6	102.1	1.0
	Galenic	143.5	0.6	144.4	0.9

[a] $n = 6$.

Accuracy was assessed by means of recovery assays at three different concentrations (5, 10 and 20 μg ml^{-1}); the mean recovery values were very good and are reported in Table 4.

3.5. Comparison of the methods

As it is emphasized in the Tables 1 and 3, both methods, and in particular the CZE method, have good precision. Moreover, the electrophoretic run is very short, lasting less than 3 min.

With regard to accuracy (Tables 2 and 4), the fluorimetric method produces satisfactory results and the electrophoretic method is even more accurate. Starting from these results, it is apparent that the amounts found declared in Prozac® capsules are very close to 100%, thus well within the prescribed range, while the galenic capsules contain much higher amounts of fluoxetine ($\sim$140%) than those declared.

The results of these assays were also compared to those obtained by means of our previously

Table 4
Accuracy of the capillary electrophoresis assay

Fluoxetine added (μg ml^{-1})	Formulation	% Recovery[a]	RSD%[a]
5	Prozac®	99.3	1.3
	Galenic	99.5	1.5
10	Prozac®	102.1	1.9
	Galenic	102.6	1.5
20	Prozac®	100.4	1.9
	Galenic	102.3	1.8

[a] $n = 6$; intermediate precision.

published HPLC method [34]. With this method, the values of fluoxetine of the declared amounts found resulted to be 100.1% for Prozac® capsules and 140.2% for galenic capsules, thus in good agreement with the values obtained by means of the fluorimetric and electrophoretic assays.

Considering these results it is thus possible to affirm that both proposed methods are fast, simple and suitable for the accurate determination of fluoxetine in commercial capsules. The electrophoretic method in particular is very accurate and precise, while the fluorimetric method is simpler and requires less expensive instrumentation.

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