ORIGINAL ARTICLE

DEVELOPMENT OF A SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF FLUOXETINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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Abstract

A sensitive and simple spectrophotometric method has been developed for the quantitative determination of fluoxetine hydrochloride in bulk and pharmaceutical formulations. The yellow complex between fluoxetine hydrochloride and yellow metanil was extracted with chloroform and its absorbance was measured at 408 nm. Beer's low limits (1.01-10.14 µg/mL), the molar absorptivity of 21028 $L \cdot mol^{-1} \cdot cm^{-1}$ and the complex composition (1:1) were determined. The stability constant, detection and quantification limits were calculated. The proposed method has been successfully applied for the assay of drug in pharmaceutical dosage forms.

Rezumat

S-a dezvoltat o metodă spectrofotometrică simplă şi sensibilă pentru dozarea clorhidratului de fluoxetină ca atare şi din forme farmaceutice. Complexul galben dintre fluoxetină şi galben de metanil a fost extras în cloroform, iar absorbanţa acestei soluţii a fost măsurată la lungimea de undă 408 nm. S-au determinat domeniul de concentraţii pentru care se respectă legea Beer (1,01-10,14 µg/mL), absorbtivitatea molară (21028 $L \cdot mol^{-1} \cdot cm^{-1}$) şi compoziţia complexului (1:1). S-au calculat constanta de stabilitate, limitele de detecţie şi de cuantificare. Metoda propusă a fost aplicată cu succes şi la determinarea substanţei medicamentoase din forme farmaceutice.

Keywords: fluoxetine hydrochloride, spectrophotometry, ion pair, yellow metanil

Introduction

Fluoxetine hydrochloride (FLX), ((RS)-N-methyl-3-phenyl-3-(4-trifluoromethyl-phenoxy)-propan-1-amine hydrochloride [21], an antipsychotic drug, acts as a selective serotonin reuptake inhibitor. The efficiency of FLX is similar of tricyclic antidepressants, being one of the most widely prescribed drugs for the treatment of major depression. The substance does not show most of the side effects of tricyclic anti-depressants being used in treating of obsessive-compulsive disorders or metabolic dysfunctions like eating disorders, for a variety of other indications as anorexia, pain associated with diabetic neuropathy and premenstrual syndrome [10].

Many analytical methods have been developed for the assay of FLX in bulk, biological samples and pharmaceutical formulations: chemical methods [4], electrochemical [14, 16], optical [6, 13, 19], chromate-graphic [1, 2-4, 7, 9, 20] etc. European Pharmacopoeia reports a HPLC method for the assay of FLX [21].

Optical methods are widely used for the assay of pharmaceutical substances and active principles of vegetable products [8, 18].

This paper describes the development of a new spectrophotometric method for the assay of FLX. The ability of this drug to form ion pairs with different dyes was studied and yellow metanil (YM) was found to be an appropriate reagent for this purpose. The experimental conditions that support the assay are also discussed. This new method was tested for the assay of FLX in bulk and pharmaceutical formulations. The results were statistically compared with those of the official method and show excellent agreement, good precision and accuracy.

Materials and Methods

Instrument: Perkin Elmer UV-VIS spectrometer Lambda 2 (Perkin Elmer) (10 mm quartz cells) for absorption spectra recording; analytical balance (Mettler Toledo AT261 Delta Range) for weighing; Metrohm 716 DMS Titrino equipped with glass combined electrode for pH measurements; ultrasonic bath Elma 9331-1 (Barnstead, Lab-Line) for dissolving.

Chemicals: Fluoxetine hydrochloride was kindly supplied by S.C. Vim Spectrum. Substance purity

was checked by determination of the melting point and the registration of IR spectrum. A 0.1% FLX stock solution was obtained by dissolving 0.1 g FLX into 100 mL distilled water. Other reagents: potassium hydrogen phthalate, NaOH, HCl conc., CH_3COOH glacial, $Na_2HPO_4 \cdot H_2O$, KH_2PO_4, Na_2SO_4 anh. (Merck KGaA, Germany), YM (Fluka) were of analytical grade. Series of solutions (0.1 M NaOH, 0.1 M HCl, 2 M CH_3COOH, 0.2 M potassium hydrogen phthalate, 1/15 M $Na_2HPO_4 \cdot H_2O$, 1/15 M KH_2PO_4 and buffer solutions of potassium hydrogen phthalate-HCl (pH = 3.0 and pH = 3.5), NaOH - potassium hydrogen phthalate (pH = 4.01, pH = 5.0), Na_2HPO_4 -KH_2PO_4 (pH = 6.5, pH = 7.4) were prepared by following adequate methods. A 0.1% YM solution into double distilled water was obtained using an official procedure [22]. Chloroform (Lach-ner) was used without previous purification. Water used throughout the experiment was double distilled.

Fluoxin® capsules 20 mg (S.C.Vim Spectrum) were assed.

Methods

Procedure for drug determination: Into a series of 100 mL separating funnels there were placed the sample solution containing 0.1-1 mg FLX, 10 mL of buffer solution of pH 3.5, 2 mL of 0.1% YM solution; the volume of aqueous phase was adjusted to 22 mL with double distilled water; added 15 mL of chloroform and the heterogeneous mixture was well shaken for 4 min and allowed to separate; the extract was transferred into 100 mL volumetric flasks through Na_2SO_4 sicc. Extraction with 10 mL chloroform was repeated 4 times and chloroform extracts were collected in the same flask. It was filled to 100 mL with chloroform. The absorbance of the organic layer was measured at 25 ± 1ºC and at λ = 400 nm in 10 mm quartz cells using a reagent blank as reference.

Pharmaceutical formulations analysis: Powder from twenty capsules containing FLX was weighed. A portion of the powder equivalent to 20 mg of FLX was accurately weighed into 50 mL volumetric flask and 30 mL double distilled water was added. The suspension was maintained in ultrasonic bath for 10 minutes and then brought with double distilled water to volume. Filtration through a Whatman No.42 filter paper was performed. Then, it has been followed the procedure for drug determination.

The composition of ion pairs was established using Job`s method of continuous variation [12] and the method was validated according to the ICH regulations Q2 (R1) [23].

Results and Discussion

Due to their sensitivity, extractive spectrophotometric methods are popular for the determination of many drugs. Ion-association spectrophotometry has received considerable attention for the assay of many drugs, both in pure form and in pharmaceutical formulations [5, 17]. FLX presents basic properties and can form protonated cations, with high molecular weight and high volume, which exhibit the ability to form ionic associations with some voluminous anions. At pH > 3, YM is present in anionic form and FLX is present in its positively charged protonated forms. When a solution of YM was added to a solution of drug at acid pH 3.5, a yellow association compound (FLX-YM) was obtained which can be extracted with chloroform.

The selection of the solvent and the wavelength: after testing several organic solvents, for extraction of the ion pair, chloroform was chosen for selective extraction of ion association from aqueous phase. The dissociation is reduced and the existence of ion pair is ensured in chloroform. The absorption spectra of the chloroformic extracts of dye, FLX and FLX - YM, were measured in the range 350-550 nm against the blank solution (Figure 1).

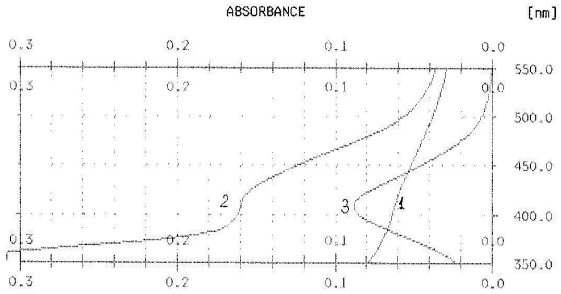

Figure 1.
Absorption spectra of extracted compounds:
FLX (1), YM (2), FLX-YM (3)

The ion-pair exhibits an absorption maximum at λ = 408 nm, which was chosen for all subsequent measurements.

Effect of time on the stability of the complex was evaluated by measuring the absorbance for a chloroformic solution at 10 minutes intervals, at 408 nm against a reagent blank. The absorbance remains stable at least 2 hours.

The optimization of the methods was carefully studied to achieve complete reaction, highest sensitivity and maximum absorbance. The effect of pH buffer, volume of the dye and shaking time on the extraction of ion-pair were studied.

The effect of pH on the ion formation was studied by extracting in chloroform the coloured complex in the presence of buffers of pH: 3, 3.5, 4.01, 6.5 and 7.4. The highest absorbance value was obtained for pH 3.5 (with 10 mL of buffer solution), which

agrees with theoretical conditions (optimum value of pH must be situated between pKa for dye and pKa for drug) and ensures maximum stability of the drug in aqueous solution (Figure 2). This value of pH was selected for further determinations.

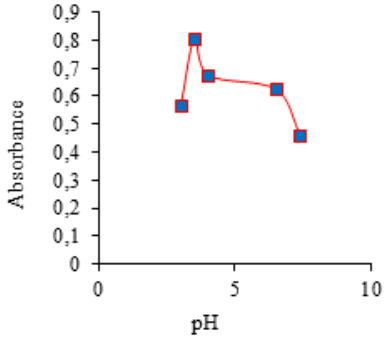

Figure 2.
Effect of pH of the aqueous solution on the of absorbance FLX-YM

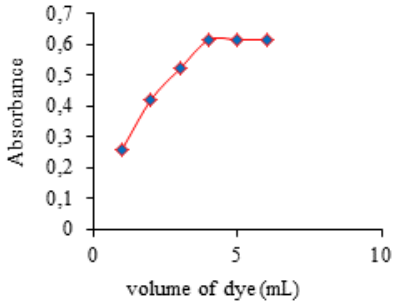

Figure 3.
Effect of the concentration of YM on the absorbance FLX-YM

The effect of the dye concentration was studied by measuring the absorbance of the organic phase after extraction from aqueous solutions containing a fixed concentration of FLX and varied amounts of dye at pH 3.5. The volume of aqueous solutions was maintained constant by adding of various volumes of double distilled water. The maximum colour intensity was achieved with 2 mL solution of 0.1% YM that provided an excess of dye (Figure 3).
Stoichiometric relationship: For the determination of molar ratio it was used Job`s method of continuous variation of equimolar solutions. There were prepared three series of solutions in which the total volume of drug and reagent (YM) was kept at 10 mL. There were used three series of standard solutions of FLX ($4.0 \cdot 10^{-4}$ M; $4.5 \cdot 10^{-4}$ M and $5 \cdot 10^{-4}$ M) and three series of solutions of YM ($4.0 \cdot 10^{-4}$ M; $4.5 \cdot 10^{-4}$ M and $5 \cdot 10^{-4}$ M). After extraction in chloroform, the absorbance was measured at 408 nm against a reagent blank. The maximum value for the absorbance was found for a ratio [drug] / ([ion pair] + [dye]) = 0.5 (Figure 4). The results indicate that 1:1 (drug: dye) ion pair was formed through the electrostatic attraction between positive protonated FLX^+ and negative YM^-. The extraction equilibrium of FLX-YM between the two phases (aqueous and chloroformic) can be represented as follows:

$$FLX^+_{(aq)} + YM^-_{(aq)} \leftrightarrows FLX^+YM^-_{(aq)} \leftrightarrows FLX^+YM^-_{(chloroform)}$$

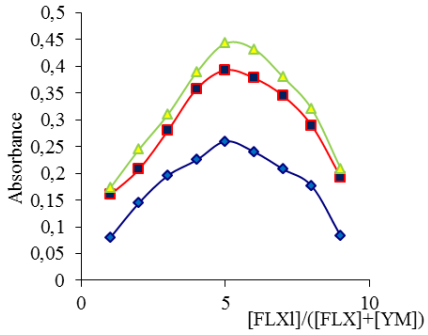

Figure 4.
Results obtained using Job`s method

The conditional stability constant (K_f) of the ion-pair complex FLX-YM was calculated from the experimental data obtained from the continuous variation method using the following equation [11]:

$$K_f = \frac{A / A_m}{[1 - A / A_m]^{n+2} C_M (n)^n}$$

(A and A_m are the observed maximum absorbance and the absorbance value when all the present drug is associated, respectively; C_M is the mole concentration of drug at the maximum absorbance and n is the stoichiometry with which dye ion associates with drug). The value for log K_f was 3 ± 0.07.
Analytical performance testing
Linearity: The standard calibration curve was constructed by plotting the absorbance *versus* concentration of FLX-YM. A linear correlation was found between absorbance at 408 nm and concentration of FLX in the concentration range 1.01-10.14 µg/mL. This was described by the regression equation, y = 0.0628x - 0.0066, where y is the absorbance of 1 cm layer of solution and x is concentration in µg/mL. The linearity of the calibration curve in the studied concentration range was proved by the correlation coefficient which was found to be 0.9996.
Sensitivity was evaluated by calculating the limit of detection (LOD) and limit of quantification (LOQ) for the proposed method using the following equation [23]:

$$LOD = \frac{3.3 \cdot s}{k} \qquad LOQ = \frac{10 \cdot s}{k}$$

where s is the standard deviation of replicate determination values under the same conditions as

for the sample analysis in the absence of the analyte and *k* is the sensitivity (the slope of the calibration curve). The values of LOD and LOQ were found to be 0.44 µg/mL, 1.34 µg/mL, respectively.

The *precision* of the assay was determined by repeatability (intraday) and intermediate precision (inter-day). Three different concentrations of FLX (4, 5 and 6 µg FLX/mL) were analysed in three independent series in the same day (intraday precision) and three consecutive days (inter-day precision). FLX concentration was calculated using the regression equation. The relative standard deviations were calculated and were found to be 0.49% for repeatability and 0.60% for the intermediate precision (Table I).

Table I

Statistical data for the calibration curve

Parameter	Value
Measuring wavelength (nm)	408
Beer`s law limits (µg/mL) *	1.01-10.14
Molar absorbtivity, ε ($L \cdot mol^{-1} \cdot cm^{-1}$)	21028
$A^{1\%}_{1cm}$	608
log K_f	3± 0.07
Regression equation (y)	0.0628x -0.0066
Correlation coefficient (r)	0.9996
LOD (µg/mL)	0.44
LOQ (µg/mL)	1.34
Repeatability (R.S.D. %)	0.49
Intermediate precision	0.60

* N = 9 (the number of calibration level), n = 3 (the number of replicates at each level)

Analysis of pharmaceutical formulations

The proposed method was successfully applied to the determination of FLX from dosage forms. Mean recovery for FLX from *20 mg Fluoxin® capsules* it was found 98.82% with a confidence range 98.82 ± 1.81 (at 95% confidence level) that showed a good agreement with the label claims. It is concluded that no interference was observed from the excipients in pharmaceutical dosage forms of FLX such potato starch and magnesium stearate, indicating high selectivity for drug.

Conclusions

The spectrophotometric methods are simple and cost efficient compared with chromatographic methods. The reagents used in the proposed method are cheaper, readily available and the procedure does not involve any critical reaction. The method is unaffected by slight variations in experimental conditions, is accurate, reproducible, adequately sensitive and free from interference by excipients. These aspects are of major interest in analytical pharmacy, offering the possibility to assay fluoxetine in its pharmaceutical dosage forms, being suitable for routine analysis in bulk and pharmaceutical formulations. Statistical comparison of the results with the official and reference methods showed excellent agreement and indicated no significant difference in accuracy and precision.

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