Spectrofluorimetric Determination of Paroxetine Hydrochloride in Its Formulations and Human Plasma

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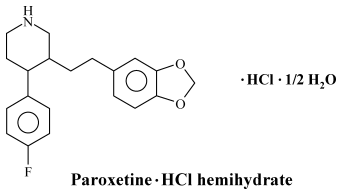
A sensitive spectrofluorimetric procedure for the determination of paroxetine–HCl in pharmaceutical formulations and human plasma has been described. The native fluorescence of the drug has been studied under different conditions. Maximum fluorescence intensity was obtained in methanol at 340 nm using 290 nm for excitation. Different surfactants showed negative effect on the fluorescence intensity of paroxetine–HCl. Regression analysis of Beer's plot showed good correlation (r=0.9999) between fluorescence intensity and concentration over the range of 0.05—0.40 μg ml^{-1} with lower limit of detection (LOD) of 0.015 μg ml^{-1}. The drug was successfully determined in its tablets with average % recovery of 98.00±0.99% which was in accordance with those given by a compendial method. The method was also applied to the determination of paroxetine–HCl in spiked human plasma with average recovery of 77.70±1.06%.

Key words paroxetine; fluorimetry; pharmaceutical analysis; spiked human plasma

Paroxetine–HCl is a new selective serotonin reuptake inhibitor used as antidepressant.[1—3)]



Paroxetine · HCl hemihydrate

Most of the reported methods for the analysis of paroxetine in biological fluids and breast milk rely on the use of chromatographic techniques including TLC,[4,5)] HPLC[6—9)] and GC[10,11)] in addition to electrophores.[12,13)] Although chromatographic methods are highly selective however, their requirements of cleaning up samples and sophisticated instrumentation preclude their use in routine analysis. Other reported methods are one voltammetric[14)] and one FIA.[15)]

Reviewing the literature revealed that, up to the present time, nothing has been reported concerning the spectrofluorimetric determination of paroxetine. This encourages the use of the native fluorescence of paroxetine to develop a simple and sensitive fluorimetric method for its determination in formulations and human plasma.

Experimental

Apparatus Fluorimetric measurements were performed using a spectrofluorimeter (Jasco model FP6200, Japan) equipped with Xenon discharge lamp and 1 cm quartz cell at medium sensitivity.

Reagents and Materials A reference standard of paroxetine–HCl was kindly supplied by Saudi Pharmaceutical Industries and Medical Appliances corporation (Buraydah, Saudi Arabia). Zeroxat C.R. tablets each labeled to contain 20 mg paroxetine as paroxetine HCl (manufactured by Smith Kline Beecham Pharmaceuticals, England) were purchased from local market. Plasma was obtained from king Khalid University Hospital, Riyadh KSA and kept frozen until used after gentle thawing.

A stock solution of paroxetine–HCl (0.5 mg ml^{-1}) in methanol (Merck, Germany) was prepared, which was found to be stable for up to 30 d if kept in a refrigerator protected from light. Working standard solution (5 μg ml^{-1}) was prepared by dilution with methanol as appropriate.

Procedure. Calibration Curve Accurate volumes (0.25—2 ml) of the working standard solution of paroxetine–HCl (5 μg ml^{-1} in methanol) were transferred into a series of 25 ml volumetric flasks and completed to the mark with methanol. The fluorescence intensity was measured at 340 nm after excitation at 290 nm. To get the calibration curve, the fluorescence intensity was plotted *versus* the final concentration of the drug and the corresponding regression equation was derived.

Analysis of Tablets Ten zeroxat CR tablets were weighed and pulverized. An accurate weight of the fine powder equivalent to 25 mg of paroxetine–HCl were transferred to a small beaker and extracted with 40 ml methanol by sonnication for 20 min, then filtered into a 50-ml volumetric flask. The flasks were completed to volume after washing the residue with methanol.

The above procedure under "Calibration curve" was then followed and the nominal contents of tablets were calculated using the regression equation.

Analysis of Spiked Human Plasma Different aliquots (0.2—0.8) ml of the working drug solution 5 μg ml^{-1} in methanol were added to 1.0 ml plasma in five standard centrifuge tubes and the volume was completed to 10 ml with methanol. Centrifugation was done at 5000 rpm for 30 min.

The fluorescence of the clear supernatant was measured at 290/340 nm. The fluorescence intensity was plotted against final concentration of the drug and corresponding regression equation was driven.

Results and Discussion

Paroxetine–HCl solution was found to exhibit an intense native fluorescence at 340 nm on excitation at 290 nm, (Fig. 1). Different media such as, water, methanol, ethanol, acetonitrile and 0.1 N H_2SO_4 were attempted. Maximum fluorescence intensity was obtained in methanol, hence it was recommended throughout this work.

The effect of different surfactants and sensitizers on the fluorescence intensity of paroxetine–HCl was studied by adding 1 ml (containing 100 μg ml^{-1}) of each surfactant solution to the methanolic drug solution (final conc. 0.25 μg ml^{-1}). It is obvious from the results, that β-cyclodextrin, gelatin and sodium dodecyl sulphate caused decreased fluorescence intensity but with the later one this decrease is dramatic. Thus, no surfactant was used in this work.

It is noteworthy to mention that although paroxetine–HCl is a relatively photolabile drug,[16)] however, no significant decrease of fluorescence intensity was observed upon exposing a 0.4 μg ml^{-1} drug solution in methanol to light from xenon arc lamp for about 4 h.

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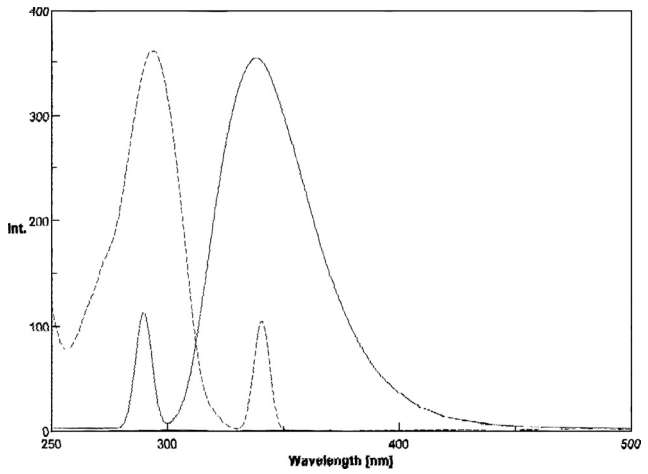

Fig. 1. The Excitation and Emission Spectra of 0.35 μg ml^{-1} Paroxetine–HCl in Methanol (λ_{ex} 290 nm, λ_{em} 340 nm)

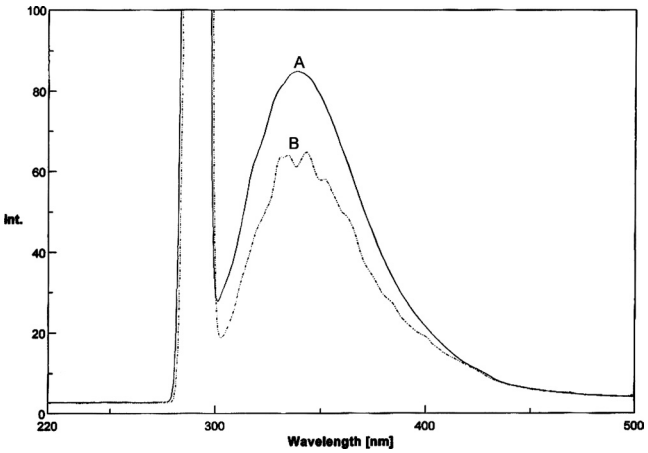

Fig. 2. The Excitation and Emission Spectra of (A) Plasma Sample Spiked with 0.1 μg ml^{-1} Paroxetine–HCl and (B) Plasma in Methanol as Blank

The validity of the method was checked by testing for linearity, specificity, precision and reproducibility.

Beer's plot was found to hold good over a concentration range of 0.05—0.40 μg ml^{-1} of paroxetine–HCl where the regression analysis of the data gave the following equation:

$$Rf = 257.94C + 0.157, \quad r = 0.9999$$

where Rf is the relative fluorescence intensity and C is the concentration of paroxetine–HCl in μg ml^{-1}.

The lower limit of detection (LOD) was found to be 0.015 μg ml^{-1}. Statistical evaluation of the regression parameters gave the following values: standard deviation of the slope (S_b) 0.23, standard deviation of the intercept (S_a) 0.06 and residual standard deviation (S_{xy}) 0.25.[17] The perfect linearity of the calibration curve is clear by the correlation coefficient.

Table 1 shows good reproducibility for the proposed procedure when applied to a pure sample of paroxetine–HCl over the range of 0.05—0.40 μg ml^{-1} (n=7). Whereas the specificity of the proposed procedure was proved by application to quantitate the drug in its tablets, confirming non-interference by excipients and additives; hypromellose, povidone, lactose, magnesium stearate, colloidal SiO_2, talc and acid copolymer dispersion.

Table 1. Application of the Proposed and a Reference Method[15] to the Analysis of Paroxetine–HCl in Pure Form and Tablets

Preparation	Recovery %	
	Proposed method	Reference method[15]
Paroxetine–HCl (pure sample)	99.6	
	102.1	
	99.5	
	99.0	
	100.0	
	100.1	
	100.4	
Mean±S.D.	100.1±0.99%	100.4±1.37%, n=5
t-value	0.143 (2.23)	
F-value	1.915 (6.16)	
Zeroxat C.R. tablets	98.8	
B.N. (20 mg paroxetine/tablet)	98.1	
	96.6	
	98.3	
	98.0±0.99%	97.9±0.67%, n=6
t-value	0.199 (2.31)	
F-value	2.183 (5.19)	

Figures in parentheses are the tabulated t- and F-values at (p=0.05).[17] Reference 15: Involves, FIA with detection at 293 nm in acetate buffer medium of pH 3.

Table 2. Application of the Standard Addition Technique to the Proposed Method in Tablets

Taken μg ml^{-1}	Added μg ml^{-1}	Found μg ml^{-1}	% Recovery of added
0.05	0.1	0.148	99.1
0.10	0.1	0.1976	98.4
0.15	0.1	0.253	102.0
0.20	0.1	0.297	98.1
0.30	0.1	0.395	98.0
Mean±S.D.			99.1±1.71%

The results were in accordance with those given by a reference method[15] as revealed by statistical analysis adopting Student's t-test and F-test where no significant difference was noticed between the two methods; (Table 1). The validity of the procedure was further assured by the recovery of standard addition, (Table 2).

The high sensitivity attained by the proposed fluorimetric procedure called for extending it to the determination of paroxetine–HCl in spiked human plasma. To avoid variation in background fluorescence, a simple deproteination of plasma samples with methanol (working solvent) has been performed followed by centrifugation and clear centrifugate containing paroxetine–HCl was analysed.

A calibration graph was first obtained by spiking plasma samples with paroxetine–HCl in the range of 0.1—0.4 μg ml^{-1}. Linear regression analysis of the data gave this equation:

$$Rf = 1.212 + 195.9C, \quad r = 0.9993$$

Where Rf is the relative fluorescence intensity and C is the concentration of paroxetine–HCl in plasma in μg ml^{-1}. The lower limit of detection was found to be 0.035 μg ml^{-1} (calculated from the standard deviation of ten readings of the blank×3). The results of analysis of 5 spiked samples of

Table 3. Application of the Proposed Method to the Determination of Paroxetine–HCl in Spiked Human Plasma

Amount added, $\mu g\,ml^{-1}$	Amount found, $\mu g\,ml^{-1}$	% Recovery
0.10	0.0767	76.7
0.25	0.1940	77.6
0.30	0.2298	76.6
0.35	0.2762	78.9
0.4	0.3144	78.6
Mean±S.D.		77.7±1.06

plasma are presented in Table 3.

Conclusion

The present work makes use for the first time of intrinsic fluorescence of paroxetine–HCl for its spectrometric determination in commercial tablets and human plasma. It is highly more sensitive than most of the reported methods. In addition, it offers simplicity, fast response and low cost.

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