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Kinetic Spectrophotometric Determination of Paroxetine Hydrochloride in Formulations and Biological Fluids

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Summary: A simple kinetic spectrophotometric method has been developed for the determination of paroxetine-HCl in bulk powder, dosage forms and biological fluids.The method is based on the oxidative coupling of paroxetine HCl with 3-methylbenzothiazolinone hydrazone / ferric chloride system in aqueous hydrochloric acid. At a fixed time of 30 min at room temperature, the formed blueish violet product was measured at 591 nm. Concentration of the drug was calculated using the calibration equation for fixed time method where good correlation was obtained in the range of 2-20 μg ml^{-1} of paroxetine HCl with a limit of detection of 0.24 μg ml^{-1} ($6.5x10^{-7}$ M). The recoveries was 100.1±0.74% and RSD was 0.37%. The procedure was successfully applied to commercial tablets with recovery of 97.6±1.07 % and the results obtained were comparable with those given by a reference method. The method could be extended to the in vitro determination of paroxetine HCl in urine and serum, with recoveries of 101.2±1.05% and 88.4±2.86%, respectively. The stoichiometry of the reaction was studied using the limiting logarithmic method and the proposed reaction pathway is presented.

Introduction

Paroxetine hydrochloride PX-HCL; 3–(1,3-Benzodioxol-5- yloxy)methyl]-4-(4-fluorophenyl) piperidine hydrochloride hemihydrate, is a selective seratonin reuptake inhibitor currently used as an antidepressant drug. Its metabolism and pharmacokinetics in humans have been extensively studied [1,2].

The reported methods for the analysis of PX-HCl were based mainly on chromatographic techniques, including HPLC[3-12], GC[13,14], TLC[15,16] and electrophoresis[17]. In addition, one voltammetric method was reported for the determination of the cited drug in human-plasma[8]. Concerning spectroscopy, the drug was assayed by flow-injection analysis (FIA) with detection at 293 nm in acetate buffer medium of pH 3 [18] and by fluorimetry in methanol at 340 nm using 290 nm for excitation[19]. 3-Methylbenzothiazolin-2-one-hydrazone (MBTH) has been frequently used for the spectrophotometric determination of several pharmaceutical

compounds, as quinolone antibacterials[20], acetaminophen and Phenobarbital[21], ritodsine and amoxicillin[22], sulfonamides[23] and josamycin[24].

In the present work, a kinetically based spectrophotometric method was developed for the determination of PX-HCl in its tablets and biological fluids through its reaction with MBTH in the presence of $FeCl_3$ as an oxidant.

Experimental

Apparatus

The spectrophotometric measurements were performed on an Ultrospec 2100 pro UV/Vis spectrophotometer (blochrom Ltd, England).

Materials and reagents

Distilled water was used allover this work.

PX-HCl standard sample was kindly supplied by Saudi Pharmaceutical Industries and Medical Appliances Corporation (Buraydah, Saudi Arabia). Seroxat tablets CR containing 20 mg PX-HCl per tablet were purchased from commercial sources. Stock solution of 0.5 mg ml^{-1} PX-HCl was prepared in distilled water. This solution was further diluted with water to give 0.1 mg ml^{-1} working solution. The stock solution was stable for about 2 weeks in the refrigerator. MBTH (Merck, Germany), 0.01M aqueous solution was freshly prepared by dissolving 0.216 gm in 100 ml water. HCl (BDH, England), 0.2 M aqueous solution. $FeCl_3$ (Surechem Products Ltd, England), 0.1M solution was prepared by dissolving 2.7gm in 0.2M HCl. Acetonitrile (Winlab, UK). Serum samples (Multi - serum - Normal, Randox Laboratories, UK). Urine samples were obtained from healthy volunteers.

Analytical procedure

Aliquots of the working solution containing 0.02-0.2 mg of PX-HCL were transferred into a series of 10-ml volumetric flasks, 2 ml of 0.01 M MBTH solution was added, followed by 2 ml of 0.1 M $FeCl_3$ solution and adjusted to volume with water. After a fixed time of 30 min, the absorbance was measured at 591 nm against a reagent blank solution. PX-HCl concentration was computed from the appropriate equation of the calibration graph for the fixed time method.

Procedure for tablets

Ten Seroxat tablets were weighed, powdered and an accurately weighed amount of powder equivalent to 5 mg of PX-HCl was transferred into a small flask and extracted with 40 ml of water by sonnication for 20 min, then filtered into a 50-ml volumetric flask. The residue was washed with few milliliters of water and filtered, washings were added to the same flask and completed to volume with water. The above procedure was then performed and the nominal content of the tablets was calculated either from the regression equation for the fixed time method.

Procedures for spiked biological fluids

Spiked urine- 2 ml human-urine in a 100-ml volumetric flask was spiked with 20 mg PX-HCl, 50 ml water was added and sonicated for 10 min then completed to volume with water. 12.5 ml of this solution was diluted to 25 ml with water to obtain 0.1 mg ml^{-1} drug solution in aqueous urine analysed as detailed under "general analytical procedure" . Blank experiments were carried out using drug-free urine sample.

Spiked serum- Into a centrifuge tube, 1 ml serum solution was spiked with 20 mg PX-HCl, 1 ml acetonitrile was added, the mixture was then centrifuged at 3000 rpm for 10 min. 0.5 ml of the clear centrifugate was diluted to 50 ml with water to obtain 0.1 mg ml^{-1} solution. Aliquots of the later solution were transferred into a series of 10-ml volumetric flasks and the above procedure was then followed. A blank experiment was performed using the same volume of unspicked serum. Absolute recovery was determined by comparing absorbances of extracted plasma or urine with those of the standard drug at the same concentrations.

Results and discussion

Second generation antidepressant drugs as PX-HCl are increasingly prescribed world-wide by psychatrics as they seem to be safer than the traditional ones. This potentiates the demand for a simple and sensitive method suitable for their routine work analysis. Thus the efficient and sensitive blueish-violet oxidative coupling

product of PX-HCl with MBTH in presence of ferric chloride in aqueous acid medium that absorbs maximally at 591 nm was used for its determination (Fig. 1).

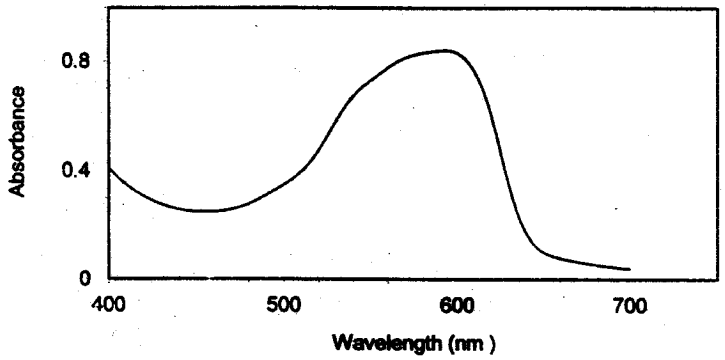

Fig.1. Absorption spectrum of the reaction product of paroxetine -HCl (12mg ml^{-1}) with MBTH-$FeCl_3$ system in HCl medium

Mechanism of the reaction

The stoichiometry of the reaction between PX-HCl and MBTH was studied by adopting the limiting logarithmic method[25]. A plot of log A versus log [PX-HCl] at a constant concentration of MBTH and $FeCl_3$ gave a straight line with a slope of 1.225 Fig. 2A). A plot of log A versus log [MBTH] at a constant concentration of PX-HCl gave a straight line with a slope of 0.456 (Fig. 2B). Thus, the molar ratio of the reaction is 1.225:0.456, which is almost 3:1 (MBTH : drug).

The reaction pathway is assumed to proceed according to the previously reported methods[20-24]; the reaction of MBTH with PX-HCl in presence of an oxidant proceeds via oxidative coupling. MBTH when oxidised with ferric ions it forms an electrophilic intermediate (E^+) which is the active coupling species. The later would be expected to attack carbon atom with maximum electron density according to the pathway shown in Fig. 3.

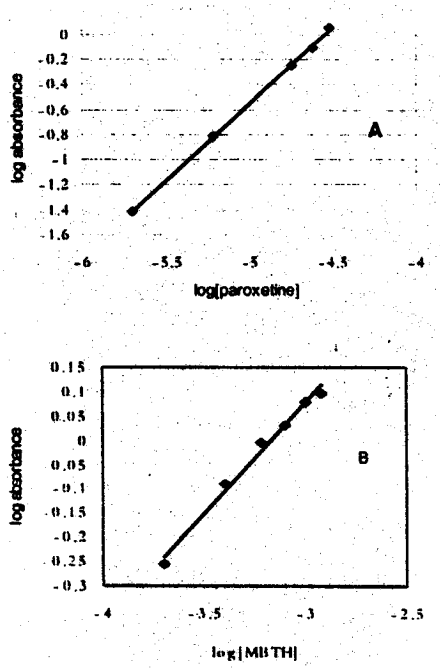

Fig. 2. Limiting logarithmic plots for determination of the molar ratio of the reaction (A) log A vs log [paroxetine HCl] at fixed concentrations of MBTH and $FeCL_3$ (B)) log A vs log [MBTH] at fixed concentrations of paroxetine HCl and $FeCL_3$

Optimization of reaction conditions

The various experimental parameters affecting the substitution reaction and stability of the colored product were studied.

The effect of MBTH concentration was studied over a range of concentration of $2x10^{-4}$-$4x10^{-3}$ M, maximum absorbance at 591 nm was obtained at $2x10^{-3}$ M after which the absorbance seemed to be constant.

Similarly, the effect of concentration of $FeCl_3$ was studied using a final concentration range of $2x10^{-3}$-$5x10^{-2}$ M $FeCl_3$. Maximum absorbance of color intensity was obtained at $2x10^{-2}$ M, after which a decrease of intensity was observed. The standing time after addition of reagents before measurements was also studied,

where a waiting time of 30 min is essential for complete color formation at ambient temperature which was stable for further 30 min.

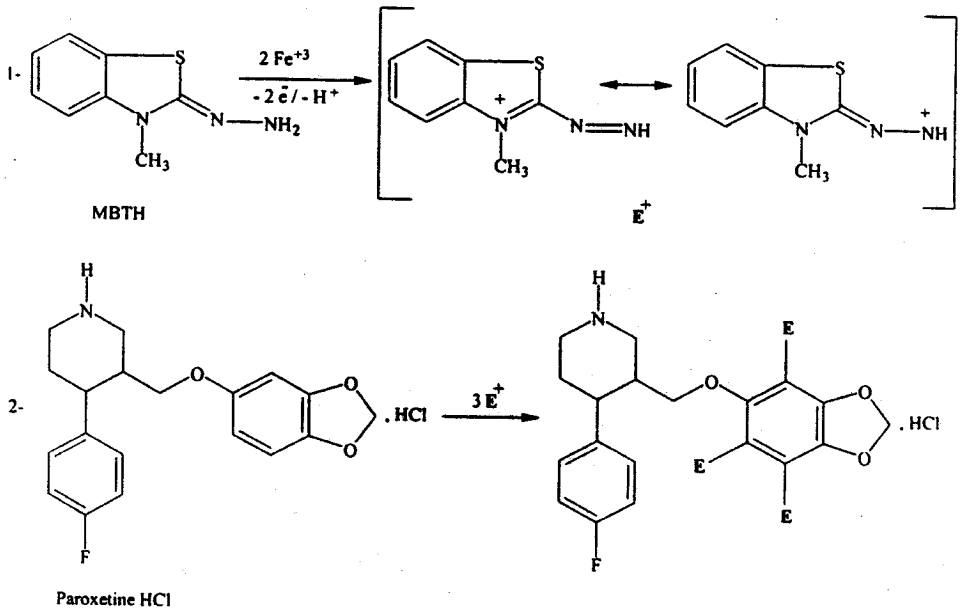

Fig. 3. The proposed reaction pathway of MBTH with paroxetine HCl in presence of $FeCl_3$ in aqueous HCl.

Various experiments were performed to study the effect of diluting solvent. PX-HCl and both MBTH and $FeCl_3$ are soluble in water and hence the reaction was firstly tried in aqueous medium then different solvents were tried as diluents namely ethanol, methanol, acetonitrile, isopropanol in addition to water. All gave comparable results to water which was preferred on the ground of cost and availability.

The effect of acidity was also investigated, although the acidity had no effect on the color development, yet it was found to be important to stabilise $FeCl_3$ solution and it was optimised by preparing $FeCl_3$ in different concentrations of HCl in the range 0.1- 2.0 M . It was found that 0.2 M is the least concentration of HCl required to stabilise the acidity of the medium.

The effect of temperature was investigated, it was found that although the substitution reaction was somewhat slow, maximum color development took about 30 min, however upon heating the reaction mixture at 90°c for 10 min and measure the brownish red color developed after further 5 min, two λ_{max} were obtained at lower wavelength 408 nm (A = 0.424) and 560 nm (A = 0.459) which were found to be about one half in sensitivity of that at 591 nm at room temperature. Thus, the reaction was carried out at ambient temperature. It was noteworthly to mention that the sequence of the reagents addition and dilution of the reaction mixture before and after the recommended time (30 min) had no effect on color development, intensity or stability of the reaction product. In addition, the reaction product development started with green color and ended with blueish-violet color. Furthermore, different oxidants were tried in the reaction instead of $FeCl_3$ as $KMnO_4$, $Ce(NH_4)_2.(SO_4)_3$, $K_2Cr_2O_7$, and H_2O_2 in acid medium. The first two oxidants gave precipitates whereas, the others gave negative effects. Thus, $FeCl_3$ was the most suitable oxidant to be used in this work.

Kinetics

The rate of the reaction was [PX-HCl] dependent where the rate was followed at room temperature at various concentrations of the drug equivalent to 2-20 μg ml^{-1} ($5.3x10^{-6}$-$5.3x10^{-5}$ M) while MBTH and $FeCl_3$ concentrations were kept constant. Fig. 4 shows that the reaction rate increased as PX-HCl concentration increased, indicating that the reaction rate obeys the following equation:

$$R = K' [PX\text{-}HCl]^n. \quad (1)$$

Where K' is the pseudo-order rate constant of the reaction.

By taking log rates and concentrations, equation (1) is transferred into:

$$\log(rate) = \log \Delta A / \Delta t = \log K' + n \log [PX\text{-}HCl] \quad (2)$$

Where A is the absorbance and t is the time in seconds .

The regression of log [PX-HCl] versus log (rate) by the least-squares yielded the following equation:

$$\text{Log rate} = -0.2155 + 0.78 \log [\text{PX-HCl}] \quad r = 0.9903 \qquad (3)$$

Thus: $K^{\backslash} = 0.61\ s^{-1}$ and the reaction is first order (n = 0.78) with respect to PX-HCl.

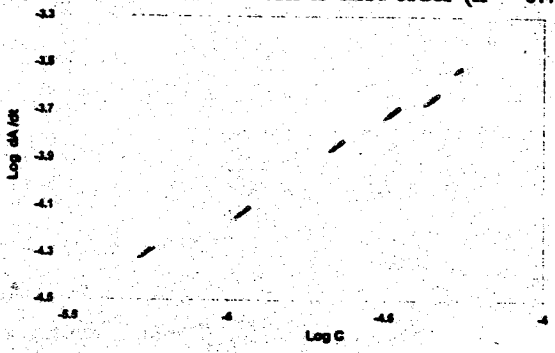

Fig.4. Relation between log rate of the reaction (dA/dt) against log concentration of paroxetine-HCl.

Evaluation of kinetic methods

The analysis of PX-HCl under the above mentioned optimum conditions where [MBTH] is about 38 times the final concentration of PX-HCl, would result in a pseudo-zero-order reaction with respect to [MBTH]. However, the rate will be directly proportional to PX-HCl concentration in a pseudo –first order reaction as follows:

$$\text{Rate} = K^{\backslash} [\text{PX-HCl}] \qquad (4)$$

Where $K^{\backslash}$ is the pseudo first order constant. Equation (4) was the basis for several experiments that were run to obtain PX-HCl concentrations using the rate data. Rate-constant, fixed-concentration and fixed-time methods [24-27] were tried and the most suitable analytical method was selected by taking into account the applicability, sensitivity, correlation coefficient (r) and intercept of the regression equations.

Rate-constant method

Graphs of absorbance vs PX-HCl concentration in the range of 1.07×10^{-5} to 5.35×10^{-5} M (4-20 μg ml^{-1}) appeared to be linear. Pseudo-first-order rate constants $K^{\backslash}$ corresponding to different drug concentrations were calculated from the slopes of log A versus t curves multiplied by -2.303 and regression of the molar concentration of the drug (C) vs $K^{\backslash}$ gave the following equation:

$$K^{\backslash} = -0.0965 + 1053.3\ C \qquad r = 0.9091 \qquad (5)$$

The value of correlation coefficient (r = 0.9091) indicates poor linearity, thus this method was abandore .

Fixed-concentration method

Reaction rates were recorded for different concentrations of PX-HCl in the range of 1.07 x 10^{-5} to 5.35x10^{-5} M. A preselected value of absorbance (0.32) was fixed and the time was measured in seconds. The reciprocal of time against the initial concentration of PX-HCl was plotted and the following equation of the calibration graph was obtained:

$$1/t = 81.67\ C + 0.00011 \qquad r = 0.9849 \qquad (6)$$

Where C is the molar concentration of PX-HCl and t is the time in seconds.

The range of concentration of PX-HCl giving the most satisfactory calibration graph with the above equation was very limited and the linearty was poor.

Fixed–time method

Reaction rates were measured for different concentrations of PX-HCl ranging from 5.35x10^{-6} to 5.35x10^{-5} M. (2-20 μg ml^{-1}) at a preselected fixed time measured accurately. Calibration graphs of absorbance vs concentration of drug at fixed times of 5-60 min were measured. The calibration equations are presented in Table (1). It was clear that the slopes increased with time and the correlation coefficient at 30 min was the highest therefore, 30 min was recommended as the most suitable time for measurements.

Determination of PX-HCl

Under the described optimum conditions, standard calibration curves for PX-HCl by the proposed method were constructed. The absorbance was linearly related to the drug concentration over the range 2-20 μg ml^{-1} with a limit of detection (LOD) of 0.24 μg ml^{-1} and a limit of quantitation (LOQ) of 0.8 μg ml^{-1}. The concentration of PX-HCl was calculated using the corresponding calibration equation presented in Table 1 at a fixed time of 30 min.

Table1. Calibration equations at different fixed times for paroxetine-HCL concentration in the range 2-20 μg ml^{-1}

Time (min)	* Calibration equation	Corrolation Coefficient (r)
5	A = 0.0296 C - 0.1084	0.9902
10	A = 0.0578 C - 0.0691	0.9940
20	A = 0.0865 C - 0.0324	0.9973
30	A = 0.0953 C - 0.0335	0.9993
40	A = 0.0931 C + 0.0176	0.9958
50	A = 0.0923 C + 0.0431	0.9946
60	A = 0.0922 C + 0.0647	0.9932

* n = 6

Statistical evaluation of regression line of this equation gave the following values: standard deviations (S.D.) of slope (S_b) and intercept (S_a) were 0.002 and 0.021, respectively. The repeatability of the propsed method was checked with 10 samples of 10 μg ml^{-1} of the drug. The relative standard deviation (RSD) was 0.37%.

The precision of the method was evaluated by analysing standard solutions of PX-HCl. The results in Table 2 were in accordance with those obtained by a reference method [18].

Analysis of tablets

The method was successfully applied to the determine PX-HCl in Seroxat tablets; revealing no interference by excipients and additives. The results in Table 2 agreed with those obtained by a reference method [18]. Statistical analysis [28] of these results using student's t-test and the variance ratio F-test showed no significant difference between the performances of the method as regards to accuracy and precision.

Analysis of spiked urine and serum samples

The high sensitivity obtained by the proposed method allows the determination of the studied drug in biological fluids. The drug can be directly analysed in spiked urine without any pretreatment. However, for human serum only a deproteination

process was carried out using acetonitrile as a sample pretreatment; an extraction procedure was not necessary. Nevertheless, for both urine and serum samples, a blank value was obtained by treating a drug free sample in the same way as described under the recommended procedure. Table 3 shows the results of the recovery studies of PX-HCl from spiked urine and serum. The low recovery obtained from serum is ascribed to the absorption of the drug on protein surface when it is precipitated[29].

Table2. Spectrophotometric determination of paroxetine HCl and its tablets

Drug form	Found (%)	
	Proposed method	Reference method [18]
Paroxetine hydrochloride		
Mean ± S. D.	100.1 ± 0.74	100.4 ± 1.37 [d]
Student's t – test	0.464 (2.262) [a]	
Variance ratio F – test	3.43 (5.19) [b]	
Seroxat tablets [c]		
Mean ± S. D.	97.6 ± 1.07	97.9 ± 0.67 [e]
Student's t - test	0.569 (2.262) [a]	
Variance ratio F - test	2.55 (5.19) [b]	

[a,b] Tabulated t and F values at (p = 0.05) [28]

[c] Smith Kline Beecham Pharmaceuticals , England .

Ref [18] Analysis by spectrophotometric method (n = 5) [d] and (n = 6) [e]

Table (3) Spectrophotometric determination of paroxetine HCl in spiked urine and serum

Concentration Taken ($\mu g\ ml^{-1}$)	Found (%)	
	Urine	Serum
4	101.4	92.7
8	101.7	85.0
12	100.5	78.0
16	102.5	89.3
20	100.0	88.1
Mean ± S.D	101.2 ± 1.05	88.4 ± 2.86

Conclusion

The results obtained in this work clearly show the usefullness of kinetic methodology for developing a simple spectrophotometric procedure for the determination of PX-HCl in dosage forms and spiked human urine and serum with good accuracy. The LOD ($6.5x10^{-7}$ M) is comparable to that obtained by the British Pharmacopoeia chromatographic method [3], however, it does not require elaboration of sample treatment or sophisticated instrumentation. The high reproducibility and sensitivity make the proposed method applicable for routine analysis of PX-HCl.

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