Bull. Pharm. Sci., Assiut University, Vol. 22, Part 1, June 1999, pp. 81-89.

UTILITY OF CERTAIN π-ACCEPTORS FOR SPECTROPHOTOMETRIC DETERMINATION OF FLUOXETINE HYDROCHLORIDE

Hesham Salem* and Mohamed F. Radwan

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Al-Azhar University, Assiut, 71524, Egypt

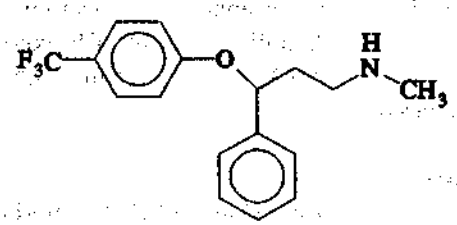
في هذا البحث تم قياس وتقدير مركب هيدروكلوريد الفلوكسيتين المستخدم علاجيا كمضاد للإكتئاب حيث تم تعيينه بإستخدام طريقة نقل الشحنات بين هيدروكلوريد الفلوكسيتين (معطى) وثلاث مستقبلات (كلورانيل وأيثيلين رباعي السيانيد وكينو ثنائي ميثان رباعي السيانيد) لتقديره كميا عن طريق تكوين معقدات ذات قوة امتصاص عالية وقد وجد أن التفاعل يتبع قانون بيير في مدى من ٠,٠٥-٣,٠٠ ملي جرام لكل ملي لتر من هيدروكلوريد الفلوكسيتين ولقد تم دراسة بعض المؤثرات مثل نسبة تركيز هيدروكلوريد الفلوكسيتين إلى المستقبلات ونسبة التركيز المؤثرة للمستقبلات ومعدل تخفيف هيدروكلوريد الفلوكسيتين وثباتية المعقد المتكون وأيضا تأثير درجات الحرارة المختلفة على المعقد المتكون. وقد تم تطبيق الطريقة المقترحة على المستحضر الصيدلي (كبسولات البروزاك) وتم مقارنة الطريقة المقترحة بطريقة منشورة فأعطت نتائج متماثلة في الدقة والتكرار.

Fluoxetine hydrochloride was determined through charge transfer complexes (CTC) formation with three electron acceptor reagents. The methods involve the reaction of Fluoxetine as n-donor with either Chloranil (tetrachloro 1,4-benzoquinone), tetracyanoethylene (TCNE) or 7,7,8,8-tetracyanoquinodimethane (TCNQ) as n-acceptors, to give a stable and highly clored radical anion. The colored products were quantified spectrophotometrically. The condition ranges adhering to Beer's law are 0.05-3.00 mg/ml. The molar ratios of the reactants were ascertained. The different parameters were carefully studied and optimized. Statistical analysis of the results revealed equal precision and accuracy to the results of the A_{max} method. The methods were applied for determination of Fluoxetine hydrochloride in pure form and in Prozac capsules.

INTRODUCTION

Fluoxetine hydrochloride is a selective serotonin reuptake inhibitor, which is clinically effective for the treatment of depression.[1] Fluoxetine hydrochloride has been shown to have comparable efficacy to tricyclic antidepressants but with fewer anticholinergic side effects.[2] Fluoxetine hydochloride has been primarily studied for the treatment of bulima and severe obesity.[3]

Different methods were applied for Fluoxetine hydrochloride determination those include chromatographic[4-8] and spectrophotometric methods.[9,10]



1-N-methyl-3-phenyl[(α,α,α-trifluoro-pathogenecity-tolyl)oxy] propylamine hydrochloride

Komoroski[11] suggested a nuclear magnetic resonance techique for analysis of Fluoxetine in human brain. Sharma *et al.*[12] introduced

Received in 5/12/1998 & Accepted in 21/3/1999

* To whom correspondence should be addressed.

spectrophotometric methods; mass, IR, NMR, for analysis of Fluoxetine. Martin and Perez[13] reported a spectrofluorimetric method for determination of Fluoxetine in which the method is based on the hydrolysis of Fluoxetine in acid medium. The fluorescent product has a spectrum with excitation and emission maxima at 253 and 306 nm, respectively. The method was applied to the determination of Fluoxetine in pharmaceutical products.

In this work, a simple, rapid and sensitive spectrophotometric method is adopted for the determination of fluoxetine hydrochloride in pure form and in Prozac capsules.

EXPERIMENTAL

Apparatus

A Shimadzu UVI601, UV-Visible Spectrophotometer (Tokyo, Japan).

Materials and reagents

The reagents used were of analytical grade and the solvents of spectroscopic grade. Fluoxetine hydrochloride formulation is marketed as 20 mg (base equivalent) capsules under the proprietary name Prozac (Lilly Co.; GB), Chloranil, TCNE and TCNQ (Aldrich Co.; USA).

Standard solutions

$5x10^{-3}$ M TCNQ, 0.2% w/v chloranil, $5x10^{-3}$ M TCNE and $4x10^{-3}$ M fluoxetine hydorchloride were prepared in acetonitrile solvent. Also the ethanolic solutions of Fluxetine hydrochloride (2 mg%) and chloranil (0.5%) were prepared.

Procedures

I- Tetracyanoqinodimethane (TCNQ) method

Accurately measured aliquots of stock solution equivalent to 0.05-0.30 mg Fluoxetine hydrochloride, TCNQ in acetonitrile (2ml) were transferred to 10 ml calibrated flasks. The contents of each flask were left to stand for 40 minutes. The absorbance of the resulting bluish green colour was measured at 843 nm, against a reagent blank prepared under the same conditions.

II- Tetracyanoethylene (TCNE) method

Accurately measured aliquots of standard solutions containing Fluoxetine hydrochloride (0.1-0.6 mg) and TCNE (6 ml) acceptor were transferred respectively to 10 ml volumetric flasks. The contents of each flask were mixed and allowed to stand for 45 minutes at room temperature. The volume was diluted to the mark with acetonitrile. The absorbance of the resulting brownish yellow colour was measured at 418 nm against a reagent blank prepared under the same conditions.

III- Chloranil methods

a) In acetonitrile

Accurately measured aliquots of solutions equivalent to Fluoxetine hydrochloride 0.4-2.4 mg, were mixed with 7 ml chloranil in 10 ml calibrated flasks. The contents of each flask were completed with acetonitrile and mixed. The absorbance of the resulting violet colour was measured at 548 nm against a reagent blank prepared under the same conditions.

b) In ethanol

Accurately measured aliquots of solutions equivalent to Fluoxetine hydrochloride 0.5-5.0 mg, in 25 ml volumetric flasks, were mixed with 2.0 ml chloranil and heated in a water bath at 50°C for 25 minutes and cooled. The contents of each flask were completed with absolute ethanol. The absorbance of the resulting colour was measured at 640 nm against blank prepared under the same conditions.

Analysis of Prozac capsules

The contents of twenty Prozac capsules were weighed and finely powdered. A portion of the powder, equivalent to 60 mg Fluoxetine hydrochloride was dissolved in 70 ml acetonitrile (by shaking for 5 minutes), filtered if necessary. The solution was completed to 100 ml with acetonitrile. Also the same procedure was done using ethanol solvent in place of acetonitrile for method IIIb. The assay was completed as under procedures I, II and III.

RESULTS AND DISCUSSION

I- Reaction with tetracyanoquinodimethane

The acetonitrile solution of Fluoxetine hydrochloride (Lewis base) when mixed with acetonitrile solution of TCNQ acceptor (Lewis acid), an intense bluish green color was developed in the visible region showing minor bands at 730, 648 and 668 n, and the major bands at 843, 825, 762 and 742 nm (Figure 1). These bands have been attibuted to the formation of TCNQ radical anion which is formed by complete transfer of n-electron from the donor Fluoxetine hydrochloride to the electron deficient π-acceptor TCNQ. The reaction may be suggested in Scheme I.

Because of the higher electron affinity of TCNQ acceptor due to the presence of four strong electrone withdrawing cyano groups in its molecule, TCNQ is very reactive as π-electron acceptor. Fluoxetine hydrochloride contains basic nitrogen with the lone pair of electrons leading to n-π-complex.[14] Beer's law was obeyed for the absorbance of Fluoxetine hydrochloride with TCNQ in the range of 0.05-0.30 mg/ml.

II- Reaction with tetracyanoethylene (TCNE)

TCNE forms CTC with n-electron donors. The presence of TCNE radical anion has been detected by optical spectroscopy showing the characteristic two λ_{max} at 400 and 418 nm (Figure 2). In most of these instances, radical formation was attributed to dissociation of the CTC with a complete one-electron transfer from the drug donor to TCNE acceptor. The proposed mechanism illustrate the complexation is in Scheme II.

The reaction mixture (donor + acceptor) was essential to attain reproducible results. The period of time allows the complete charge of the molecular complex (outer complex) into the inner complex having radical ions formation, which is responsible for the observation of λ_{max} produced. A linear relationship was TCNE in the concentration range 0.1-0.6 mg. This has indicated the very high sensitivity of the proposed procedures.

III- Reaction with chloranil

a) In Acetonitrile

Mixing the acetonitrile solution of Fluoxetine hydrochloride with chloranil gave a violet color of high absorption intersity at λ_{max} 548 nm (Fig. 3). The formed new band was attributed to an electron transfer complexation reaction between fluoxetine as n-donor and chloranil as electron acceptor followed by formation of radical ions. The proposed mechanism illustrated the complexation reaction is in Scheme III.

One mole of chloranil is consumed in the reaction with fluoxetine hydrochloride to form the radical anion, which is responsible for the produced violet color. This finding has been expexted as the cited drug contain only one basic center in their molecules, which participated in CTC formation.

b) In ethanol

Fluoxetine hydrochloride have a secondary amino group in which it can act as an electron donor and participate in chargetransfer complexation with chloranil acceptor. On studying the absorption curves for fluoxetine hydrochloride, chloranil, and fluoxetine hydrochloride chloranil charge transfer complex, λ_{max} was exhibited at 640 nm. Figure 4 indicate the formation of charge transfer complex. A linear relationship was obtained for the absorbance of chloranil with fluoxetine hydrochloride in the concentration range, 0.4-2.4 mg in the final measured solution.

IV- Investigation of assay parameters

1- Molecular ratio of the reactants

On studying the molar ratio of fluoxetine hydrochloride with chloranil or TCNQ or TCNE by both the continuous variation and mole ratio methods[15], it was found to be 1:1.

2- Effect of variation of acceptor concentration

It was found that, the most suitable volume for carrying out the assay was 2,6,7 and 2 ml of TCNQ, TCNE, chlorani (in acetonitrile) and chloranil (in ethanol), respectively with fluoxetine hydrochloride.

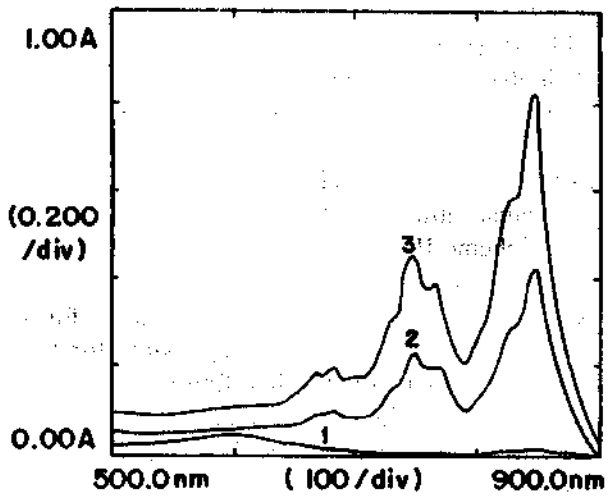


Fig. 1: Absorption spectra of fluoxetine HCl (0.15 mg) 1, TCNQ 2 and fluoxetine-TCNQ CTC 3 in 10 ml acetonitrile.

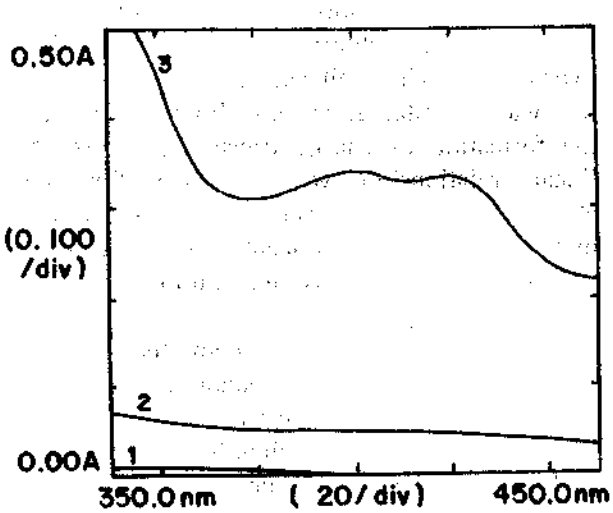


Fig. 2: Absorption spectra of fluoxetine HCl (0.30 mg) 1, TCNE 2 and fluoxetine-TCNE CTC 3 in 10 ml acetonitrile.

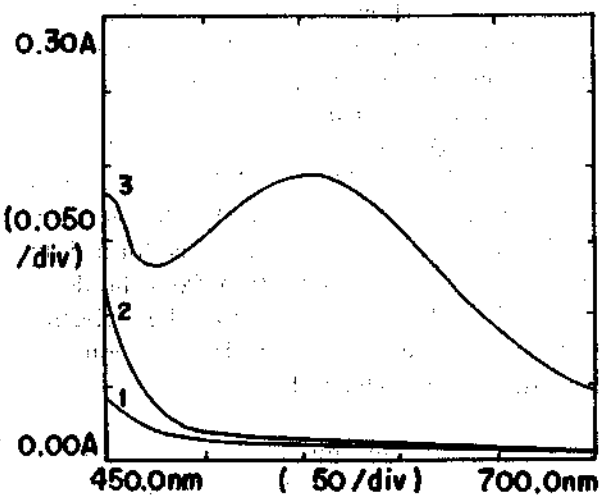


Fig. 3: Absorption spectra of fluoxetine HCl (1.2 mg) 1, chloranil 2 and fluoxetine-chloranil CTC 3 in 10 ml acetonitrile.

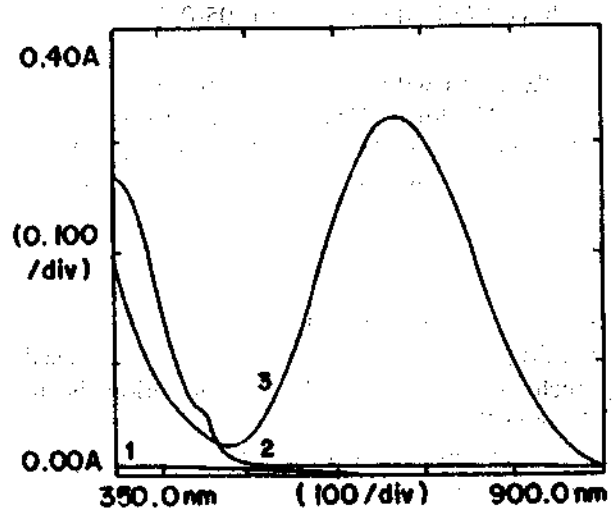
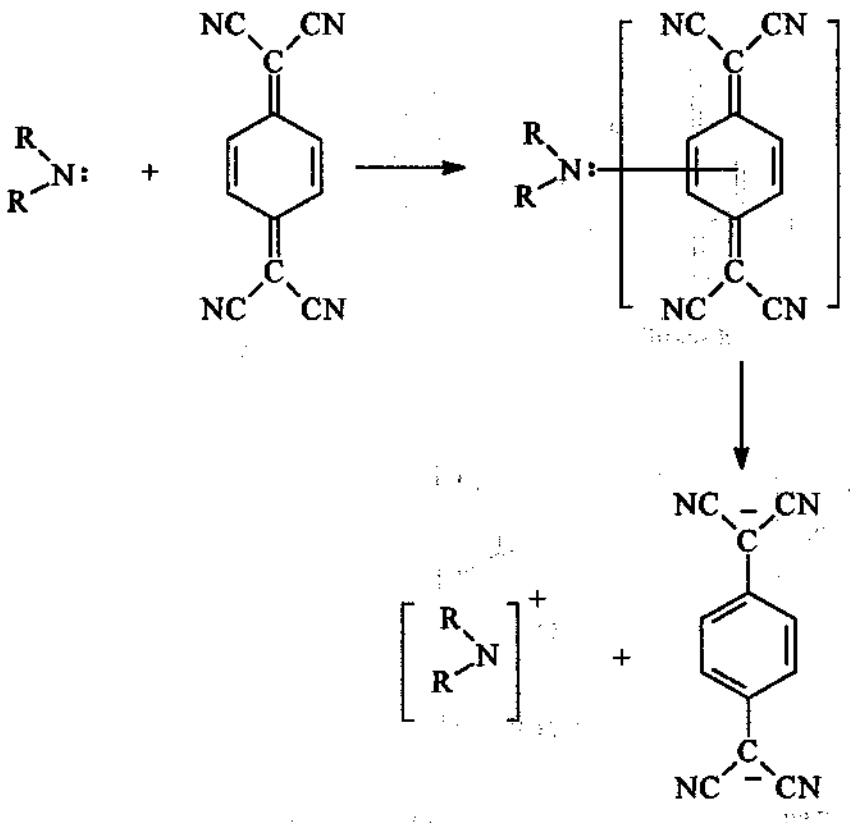
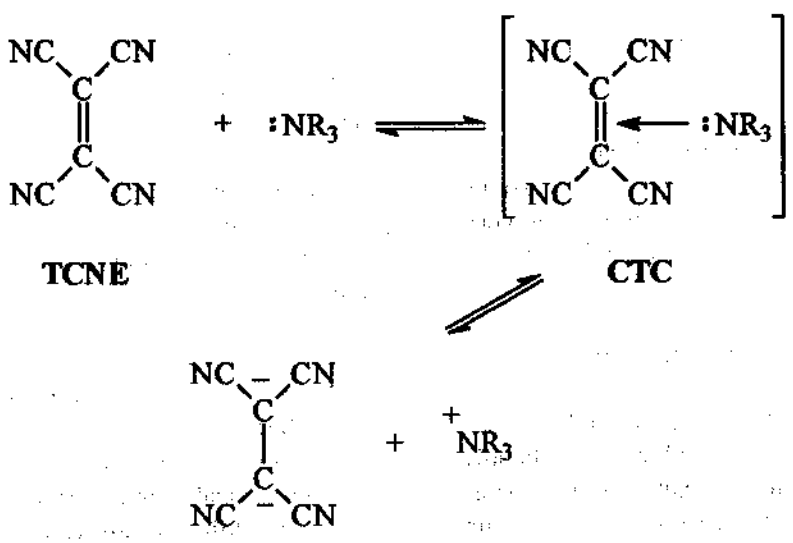


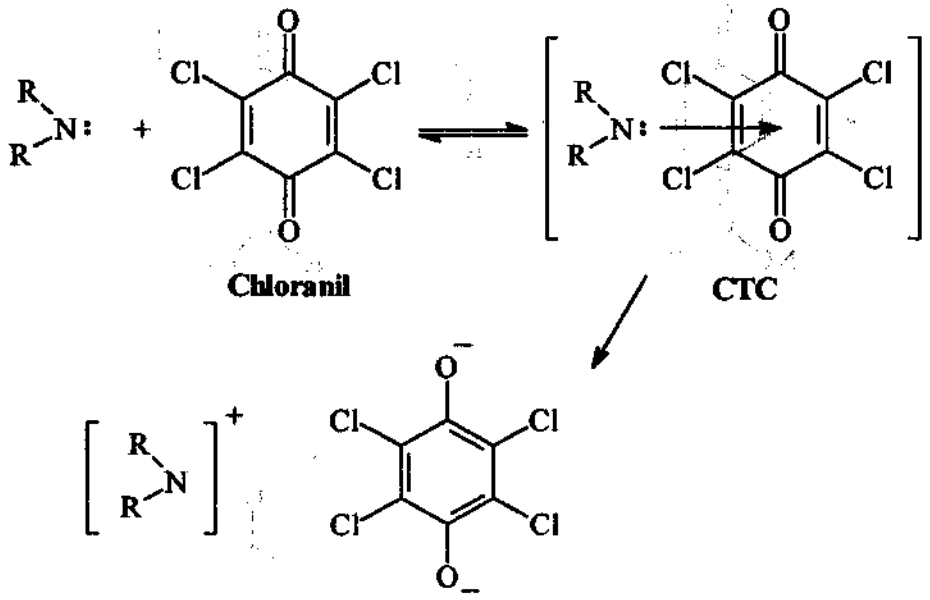
Fig. 4: Absorption spectra of fluoxetine HCl (1.5 mg) 1, chloranil 2 and fluoxetine-chloranil CTC 3 in 10 ml ethanol.



Scheme I



Scheme II



Scheme III

3- Effect of diluting solvent

Different solvents were tried for CTC formation. Acetonitrile was the solvent of choice with respect to reaction speed, stability and high sensitvity. It is a suitable medium for electron transfer complexation reaction leading to radical ion formation. Also, it was found to be a suitable solvent for TCNQ.

In case of procedure IIIb, ethanol is the most suitable one.

4- Stability of the reaction product

The produced colour of fluoxetine hydrochloride with selected acceptors was very stable for 45 minutes at least, without any change of absorbance.

5- Effect of heating temperature and heating time

It was found that heating at 50°C for 20 minutes for procedure IIIb (chlorani in ethanol) produce maximum color intensity, on increasing the temperature of the reaction, the colour intensity of complex decreased.

6- Comparison and statistical analysis

The optimum parameters for the assay of fluoxetine hydrochloride are presented in (Table. 1). The validity of the proposed procedures for determination of fluoxetine hydrochloride in pure state and in prozac capsules (Table. 2) was tested by analyzing these products with the proposed procedures and compared with A_{max} method.[16]

Statistical analysis of the results obtained indicated that the proposed procedures were accurate and precise as the A_{max} method.[16]

A standard addition technique was used for the determination of fluoxetine hydrochloride in prozac capsules to give the percentage recovery of an added amount (Table. 3) in which no interference from execpients and vehicle was encountared.

7- Effect of some additives on the proposed method

Before dealing with the analysis of the pharmaceutical preparation, the effect of common additives, adjuvants and excepients on the proposed method were experimentally studied. The results obtained in (Table. 4)

Table 1: Optimum parameters for calibration curves comstruction.

Method	Conc. range Mg/ 10ml	Solvent	No. of Experiments	Regression Analysis		
				B	K	R
TCNQ	0.05-0.30	acetonitrile	6	0.0081	3.8651	0.9999
TCNE	0.10-0.60	acetonitrile	6	0.0099	4.7791	0.9998
Chloranil	0.40-2.40	acetonitrile	6	-0.0079	8.3971	0.9999
Chloranil	0.50-3.00	ethanol	6	0.0080	6.9131	0.9999

B, intercept K, slope R, correlation coefficient

Table 2: Statistical analysis of the results optained for assay of Fluoxetine hydorchloride (authentic and in Prozac capsules) using the proposed methods compared with the Amax method.

		Amax	TCNQ	TCNE	Chloranil in CH_3CN	Chloranil in ethanol
Authentic Fluoxetine hydrochloride	$\bar{X}$	100.00	100.10	100.20	99.90	99.8
	±S.D	0.90	0.85	0.85	0.87	0.89
	N	6	6	6	6	6
	V	0.81	0.72	0.83	0.76	0.79
	t(3.58)		0.19	0.18	0.15	0.43
	F(4.28)		1.13	1.02	1.07	1.03
Fluoxetine hydrochloride in Prozac capsules	$\bar{X}$	100.00	100.2	100.1	99.8	100.1
	±S.D	0.90	0.88	0.92	0.87	0.91
	N	6	6	6	6	6
	V	0.81	0.77	0.85	0.76	0.83
	t(3.58)		0.83	0.19	0.38	0.19
	F(4.28)		1.05	1.05	1.07	1.02

Table 3: Assay of prozac capsules using the suggested CTC methods applying the standard addition techniques.

	TCNQ	TCNE	Chloranil/CH_3CN	Chloranil/ethanol
$\bar{X}$	99.8	99.9	99.8	100.0
±S.D	0.87	0.80	0.79	0.88

Table 4: Effect of some common additives, adjuvants and exepients on the proposed method.

Fluoxetine hydrochloride (mg tagen)	Other ingredients (mg added)	Fluoxetine hydrochloride (mg found)	Recovery %
1.0	----------	1.000	100.0
1.0	2 mg Strach	0.997	99.7
1.0	2 mg Talc	0.995	99.5
1.0	2 mg Glucose	0.998	99.8
1.0	2 mg Lactose	0.991	99.1
1.0	2 mg Mag. stearate	1.004	100.4

revealed that glucose, lactose, talc, magnesium stearate and starch do not interfere.

As conclusion, the proposed method can be considered simple, rapid, sensitive and selective ones for routine analysis of Fluoxetine hydrochloride either in raw material or in its dosage form.

REFERENCES

1- P. Stark and C. D. Hardison, J. Din. Psychiatry, 46 (3), 53 (1985).
2- P. Benfield, R. C. Heel and P. S. Lewis, Drug, 32, 481 (1985).
3- C. P. I Freeman and M. Hamposn, Int. J. Obesity, 11 (3), 171 (1987).
4- S. Piperaki, S.G. Penn and K. M. Goodall, J. Chrom. 700 (1-2), 59 (1995).
5- S. Pichini, R. Pacifici, Altieri, M. A. Pellegrini and P. Zuccaro, J. Liq. Chrom., 19 (12), 1927 (1996).
6- S. Piperaki and M. Parissipoulou, Ibid., 19 (9), 1405 (1996).
7- D. D. Wirth, B. A. Olsen, D. K. Hallenbeck, M. E. Lake, S. M. Gregg and G. M. Perry, Chromatographia, (9-110), 511 (1997).
8- P. D. Fergusa, D. M. Goodall and J. S. Loran, J. Chromatography, 768 (1), 29 (1997).
9- G. Fronza, G. Fuganti and P. Grasselli, Tetrahedron, 4 (8), 1909 (1993).

10- A. Hammadi and C. Croazel, J. of Radiopharm., 33 (8), 703 (1993).
11- R. A. Komoroski, Anal. Chem., 66 (20), 1024 (1994).
12- V. L. Sharma, K. Bhandari and C. M. Sing, Indian J. of Chem., 34 (11), 1000 (1995).
13- M. I. G. Martin and C. G. Perez, Anal. Lett., 30 (14), 2493 (1997).
14- R. Foster, "Organic Charge Transfer Complexes", Academic press, London and new York (1969).
15- J. Rose, "Advanced Physico-Chemical Experimental", Pittman, London, p.54 (1964).
16- "Analytical Profile of Drug Substances", Academic Press, New York, vol. 118, 205 (1987).