OPTIMIZED AND VALIDATED SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF CLOMIPRAMINE AND PAROXETINE HYDROCHLORIDE IN DRUG FORMULATIONS

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Abstract

Two simple and sensitive spectrophotometric methods have been developed for the determination of some anti-depressant drugs such as clomipramine (CLO) and paroxetine (PRX). The methods involved the formation of ion-pairs between the inorganic complexes of molybdenum(v) thiocyanate and hexakis iron(III) solution followed by extraction with 1,2-dichloroethane. The optimum conditions for the ion-pairs formation were established under which Beer's low was obeyed for CLO and PRX in the concentration range 5-150 and 10-250 μg mL^{-1} for the first method, while it was obeyed for the second method in the concentration range of 10 - 200 μg mL^{-1} for both CLO and PRX drugs, respectively. The limits of detection were 0.0744 and 0.109 μg mL^{-1} and 0.177 and 0.113 μg mL^{-1} for CLO and PRX using the first and second methods, respectively. The limits of quantification for the first method were 0.223 and 0.531 μg mL^{-1} while they were 0.327 and 0.340 μg mL^{-1} using the second method for CLO and PRX drugs, respectively. Both of the two methods have been successfully applied for the determination of the cited drugs in row materials and in drug formulations and compared with the official reference methods. Complete validation of the proposed methods was done.

Keywords: ion-pair, molybdenum(V)-thiocyanate, hexakis iron(III)-thiocyanate, clomipramine, paroxetine.

Introduction

Clomipramine hydrochloride is 3-chloro-5-[3-(dimethylamino) propyl]-10, 11-dihydro-5H-dibenz-[b, f] azepine monohydrochloride and paroxetine is (3S, 4R)-3-[(1,3-benzodioxol-5-vloxy)methyl-[4-(4-flurophenyl) piperidine. They are typically anti-depressant drugs, and widely used in the treatment of mood disorders, particularly depression and anxiety disorders. Several spectrophotometric methods have been reported for the determination of clomipramine hydrochloride [1-6]. It could be also determined electrochemically using different techniques [7-9]. Chromatographic techniques were suggested for the determination of the drug in

row materials and in biological fluids using HPLC and different detectors [10-14]. Fast LC-MS/MS method [15], capillary gas chromatographic method with flame ionization detection was also used [16] as well as Capillary zone electrophoresis methods [17, 18]. Paroxetine was determined by different methods such as chromatographic methods [19-25] and electrochemical methods [26, 27]. Paroxetine was determined spectrophotometrically via charge transfer complex formation with TCNQ and other reagents [28-30]. This work was undertaken in order to study the analytical aspects of the reaction between the drugs under investigation with molybdenum(V)–thiocyanate and hexakis iron solutions. This also aims to test the sensitivity, accuracy and selectivity of the ion-pair formation methods to use them for the determination of the cited drugs in row materials and pharmaceutical preparations. The structures of CLO and PRX are shown in Figure (1).

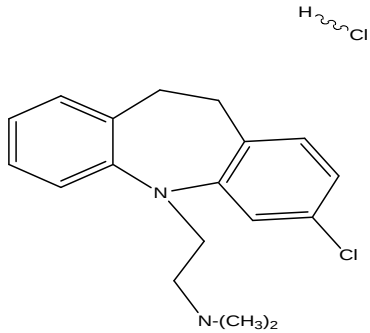

Fig. (1): Structures of CLO and PRX drugs.

Experimental

Apparatus

A Perkin-Elmer spectrophotometer model 601 with matched quartz cell of 1.0 cm optical path length was used for spectrophotometric measurements at the wavelength range of 350-6oo nm.

Materials and Solutions

All reagents were of analytical grade and used without further purifications. The water used was always doubly distilled. CLO row material was supplied from Acapi

Co., Egypt. Anapramine 75 mg/tablet was supplied from Sigma pharmaceutical industries, Egypt. Anafranil 25 mg/tablet was supplied from Novartis, Egypt.

PRX row material was supplied from Elpheronea Co., Egypt. Paroxetine and xanadole (20 mg /tablet) were supplied from Eva pharma. and European Egyptian Pharm, Egypt, respectively. Their solutions (1mg mL^{-1}) were freshly prepared by dissolving the drugs in methanol and kept at 4 ºC, in PVC containers, in the refrigerator. Other solutions were prepared by appropriate dilutions. Stock Mo(VI) solution (0.02% w/v) was prepared from AR grade ammonium molybdate in doubly distilled water containing a few drops of ammonia and standardized gravimetrically using 8- hydroxyquinoline [31]. Ammonium thiocyanate and ascorbic acid solutions (10% each) were prepared in doubly distilled water. Hexakis (thiocyanato) iron(III) solution (0.05M) was prepared by dissolving 1.34 g of $FeCl_3.9H_2O$ and 5.87 g of NH_4SCN in 100 ml water (pH 1.8).

Tablets solutions

Ten tablets of CLO or also another ten of PRX were accurately weighed and the average weight of tablets was calculated. The tablets were crushed well to a fine powder. A weighed portion of a finely ground powder equivalent to the calculated weight of pharmaceutical preparations was dissolved in ethanol. The solution was then filtered through 0.45 µm millipore filters, in order to separate out the insoluble excipients, and washed with the specific solvent. The filtrate and the washings of drugs were collected in 100 ml measuring flask. The solution was directly analyzed, according to the general analytical procedures without the necessity for sample pretreatment.

Procedure

Determination of CLO and PRX via ion pair formation with Mo(V) thiocyanate

2 mL of 0.02% (w/v) of ammomium molybdate, 2 mL HCl (4 M), 2 mL (10% w/v) each of ammonium thiocyanate and ascorbic acid were placed in a 50 ml capacity separating funnel. The mixture was left for 15 min at room temperature (20 ±1 ºC). Different volumes of 1 mg mL^{-1} of CLO or PRX solutions (0.2 – 2.5 mL) were added and diluted with bidistilled water up to 20 mL. After 20 min., 10 mL of 1,2-dichloroethane was added (twice with 5 ml portions). The mixture was shaked well for 1 min and allowed to stand to separate into two phases. The ion pair was extracted with 1,2-dichloroethane (2 × 5 ml) and the absorbance of the filtered

extract was measured at 470 nm for both CLO and PRX, respectively, against a reagent blank, which prepared similarly without the drug.

Determination of ClO and PRX via ion pair formation with hexakis(thiocyanato) iron (III) solution.

Aliquots of drug solutions (1mg mL^{-1})were transferred in 50 mL separating funnel, 2 mL of 0.05 M $Fe(SCN)_6^{-3}$ carrier solution, 2 mL of HCl (10 M) were added and the solution diluted with distilled water up to 10 mL, the mixture was left for 5 min at room temperature (20±1 ºC). 10 mL of 1,2-dichloroethane was added (twice with 5 ml portion). The mixture was shaked well for 1 min and allowed to stand to separate into two phases. The ion pair was extracted with 1,2-dichloroethane (2 × 5 ml) and the absorbance of the filtered extract was measured at 500 nm for CLO and PRX, respectively against a reagent blank prepared similarly without addition of drugs.

Results and discussion

This work is undertaken in the view that ion-pairs are formed between the tertiary amino group of PRX and CLO drugs and molybdenum(V)- or iron(III)-thiocyanate binary complexes via the protonated nitrogen atom of the drugs [32]. Molybdenum(V) formed by the reduction of Mo(VI) with ascorbic acid, combines with ammonium thiocyanate to form a red Mo(V)-thiocyanate binary complex in hydrochloric acid solution. On adding CLO or PRX solutions, orange red ion pairs are formed in the same acid concentration. The ion pairs are soluble in 1,2-dichloroethane while the binary complexes are insoluble. A double extraction is necessary to extract the ion pairs quantitatively into the organic phase. It was found that, the reduction probability of Mo(VI) to Mo(V) may occur by ascorbic acid or SCN^- in acidic media [33]. However, the rapidity, sensitivity and stability of Mo(V)-thiocyanate binary complex are enhanced by using ascorbic acid. Ascorbic acid gives reproducible values and masks many of interfering ions [33]. From the data shown in Fig. (2) it is found that $16.8x10^4$ µg mL^{-1} ascorbic acid is sufficient for the reduction of 56.6 µg mL^{-1} of Mo(VI) to Mo(V). The addition of excess amount of ascorbic acid, more than the required volume, has no effect on the absorbance of the ion-pairs formed. It was found that the ion pairs were formed only in hydrochloric acid, sulfuric, nitric or phosphoric acid medium, but the absorbance readings of 1,2-

dichloroethane extract in HCl (4 M) medium has been selected as the suitable medium for ion pair formation [34]. 4.0 mL of 4 M HCl is suitable for the formation of Mo(V)-thiocyanate-CLO and Mo(V)-thiocyanate-PRX ion pairs. The effect of ammonium molybdate on the ion pair formation shows that 3.5 ml of ammonium molybdate is required for maximum absorbance in a final volume of 20 mL aqueous solution. Also it was found that 2.5 and 4.5 mL of 10% (w/v) ammonium thiocyanate in a final solution of 20 mL gave the maximum absorbance for the determination of the cited drugs. In this method the complete formation of the ion pairs needs 20 min before extraction with 1,2-dichloroethane at 25 ºC. The absorbance of Mo(V)-thiocyanate binary complex is stable after 15 min while Mo(V) thiocyanate drug ion pairs need 20 min for their complete formation Fig. (4). The stoichiometry of Mo(V) to each drug in the presence of excess amount of ammonium thiocyanate was determined by continuous variation method. The results indicate that 1:1 metal : drug ion-pairs are formed through the electrostatic attraction between positive protonated drugs and thiocyanate negative complex as shown by the proposed structures I and II as indicated in the Fig. (5).

The effect of time on the formation of the ion pairs formed between hexakis (thiocyanato)–iron(III) and PRX and CLO drugs was studied carefully and indicated that the ion pairs attain high absorbance for PRX and CLO at 1-5 minute and decrease slowly tell remain constant and after 30 minute increase slowly and remain unchanged with increase time of mixing. Absorbance-temperature curve represents the reaction of CLO or PRX with iron(III) thiocyanate at λ_{max} = 500 nm at temperature range from 5 to 60 ºC Fig. (6). This figure shows that the absorbance is generally increased by temperature increases and reached a maximum value at 20-25 and 2o-30 ºC for CLO and PRX, respectively, and decrease slowly then become constant above this temperature. Therefore, the temperature chosen was (25± 5) as the best temperature range for determination of CLO and PRX, respectively, in pure band pharmaceutical forms. The nature of binding of iron(III) to each drug in the presence of ammonium thiocyanate was determined by continuous variation method to check the ratio between iron (III) and CLO and PRX Fig. (7) indicate that 1:1 [iron(III)] : [drug] ion pairs were formed through the electrostatic attraction between positive protonated drugs, CLO^+, PRX^+ and thiocyanate negative complex $[Fe(SCN)_6]^-$ .Solvents like acetone, pentanol, n-butyl and ethyl alcohol, 1,4-dioxane, acetone, dimethylformamide, carbon tetrachloride, diethylether and petroleum ether can not be used for the extraction of the formed ion-pairs while methylene chloride

and 1,2-dichloroethane extract these ion pairs quantitatively. The molar absorptivity values for the ion-pairs in 1,2-dichloroethane are 1.79×10^3 and 1.97×10^3 $l.mol^{-1}cm^{-1}$ at λ_{max} 500 nm for CLO and PRX, so it is selected as the best medium for extraction. Reproducible absorbance readings were obtained after either single or double extractions with 10.0 mL of methylene chloride and 1 min shaking time. The studied ion-pairs are stable for more than 1 weak at 25 ºC in the organic solvents.

Analytical data and methods validation

In order to prove the validity and the applicability of the proposed methods and the reproducibility of the results obtained, five replicates experiments at four concentrations of CLO and PRX were carried out. Table (1) shows the values of the between day relative standerd deviations for different concentrations of the drugs, obtained from experiments carried out over a period of 4 days, it was found that the between day relative standard deviations were less than 1.0% which indicates that the proposed method is highly reproducible and Mo(V), iron(III) thiocyanate binary complexes are successfully applied to determine CLO and PRX via formation of ion pairs. Under the optimum conditions described above, the calibration graphs were constructed for both drugs. The molar absorptivity, Sandell sensitivity and regression equation obtained by the method of least square treatment of calibration data (n = 5) for each drug were tabulated in Tables (2) together with Beer's low limits, correlation coefficient, detection and quantification limits and standard deviations (SD). The correlation coefficient of the data obtained are 0.94 and 0.99 for CLO and PRX using Mo(V) thiocyanate and 0.993 and 0.998 using iron(III) thiocyanate, respectively. The standard deviations are found to be 0.395-0.919 and 0.127- 1.03 and 0.33-0.49 and 0.179-0.894 while the relative standard deviations are 0.396-2.88, 0.637-0.751 and 0.123-1.67, 0.569-0.902 for CLO and PRX using Mo(V) and iron(III) thiocyanate, respectively, for five replicate determinations. The low values of the relative standard deviations indicate the high accuracy, reproducibility and sensitivity of the methods. The regression equation associated with the calibration plots exhibited good linearity which supported the validation of the proposed procedure for the quantitation of the drugs. LOD and LOQ were calculated using the relation K (SD_a)/b where K = 3 for LOD and 10 for LOQ [35] valued given in tables (2) confirming the high sensitivity of the proposed procedure compared to the official methods [36] .The repeatability of the methods were determined from multiple measurements at each of the studied samples by

performing five replicates measurements (n = 5). A mean recovery of 99 ± 0.5 was achieved, which indicates a high precision of the proposed procedure for assay of the drugs.

Application:

The validity of the proposed method was tested by determination of CLO and PRX in dosage forms manufactured in the local companies. The concentration of the drugs in the dosage forms was calculated from the appropriate calibration graphs. There was no shift in the absorption maximum due to the presence of other constituents of the dosage forms. Table (3) shows the results obtained during the determination of CLO and PRX in the dosage forms. The results are compared with those obtained applying the official method. The results obtained were compared statistically by t–test and F–test with those obtained by official method on the sample of the same batch. The t–test and F–values obtained at the 95% confidence level and degree of freedom did not exceed the theoretical tabulated value indicating that there is no significant difference between accuracy and precision of the proposed and the official methods. The correlation coefficient values obtained are 1.0 and 0.9995 using Mo(V)-thiocyanate and 0.9995 and 0.9994 using iron(III)-thiocyanate for CLO and PRX drugs, respectively, indicating the possibility of successful applications of the proposed methods in routine analysis.

Interferences

Interferences are mainly basic compounds that contain hetero nitrogen atoms in their aromatic nuclei; however, such compounds are not usually present with the examined drugs in pharmaceutical preparations and hence are not likely to cause analytical problems. On the other hand, tablets fillers such as lactose, starch, stearic acid and preservations used in their preparations which can represent a potential source of interference in other methods don’t interfere in the proposed method. That is to say the proposed method can be considered to be selective.

Conclusion:

In conclusion, simple, sensitive and selective methods are reported for the determination of CLO and PRX drugs in bulk form and pharmaceutical preprations. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the method. From the calculated t and F values, it is clear that the results obtained are in good agreement with those obtained by official method.

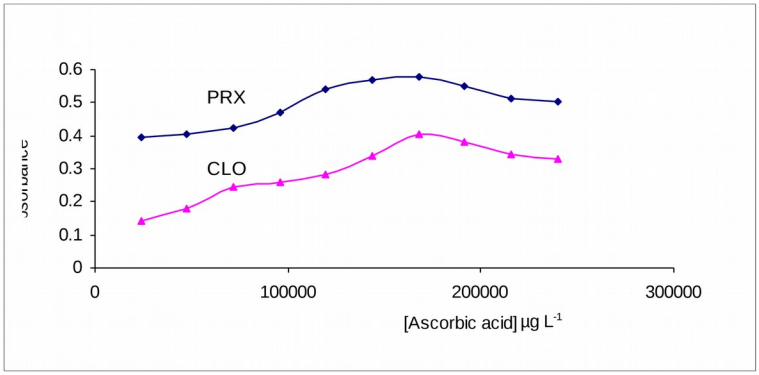

Fig. (2). Effect of ascorbic acid.

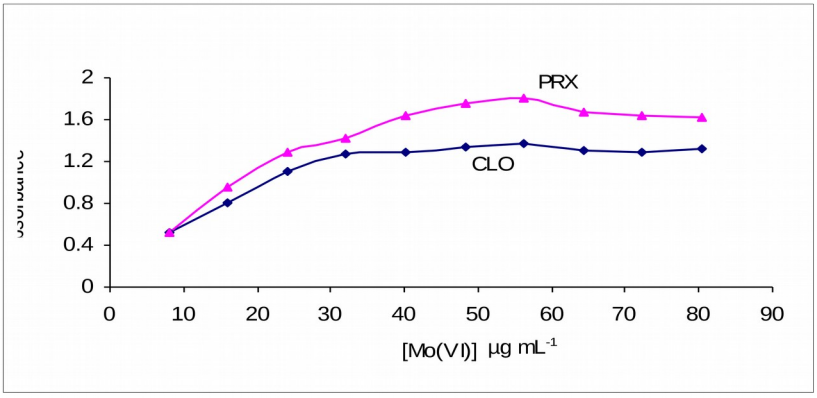

Fig. (3). Effect of Mo(V) ion concentration

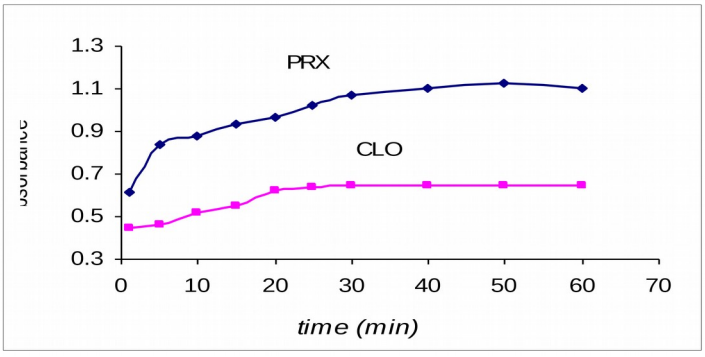

Fig (4) Effect of time on the spectra of the ion- pairs at λ_{max} = 470 nm.

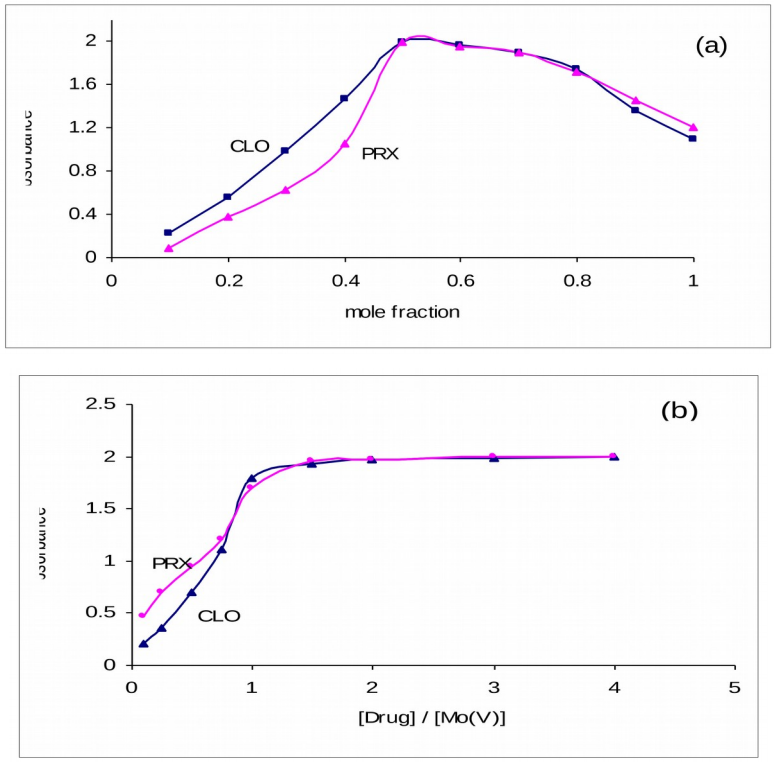

Fig. (5). Stoichiometric ratio of the reaction of Mo(V)-thiocyanate with CLO and PRX drugs using (a) continuous variation and (b)molar ratio methods at λ_{max} = 470 nm.

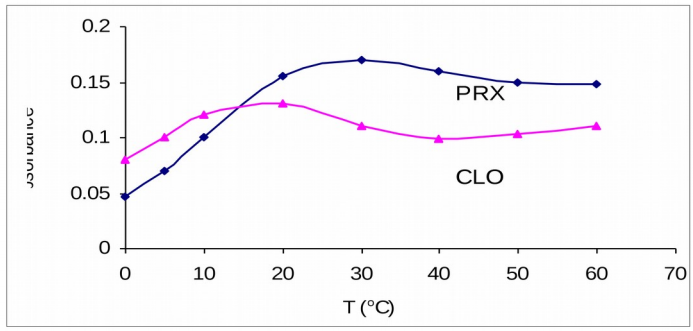

Fig. (6). Effect of temperature on formation of ion pair.

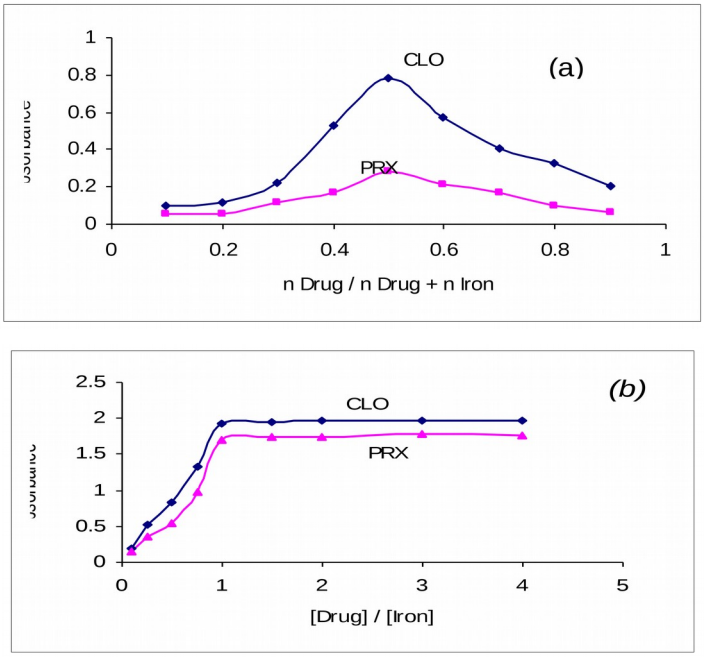

Fig. (7) .Stoichiometric ratio of the reaction of iron(III)-thiocyanate with drugs using (a) continuous variation and (b)molar ratio methods at λ_{max} = 500 nm.

The structure the ion pair:

I- $[Mo(SCN)_6]^-$-CLO ion pair II-$[Mo(SCN)_6]^-$-PRX ion pair.

Table (1). Between – day precision of the determination of CLO and PRX by the proposed method using Mo(V)-thiocyanate and iron(III)-thiocyanate.

Compound	[Drug] taken μg mL^{-1}	[Drug] found μg mL^{-1}	Percent Recovery (%)	SD	RSD
I- Using Mo(V)-thiocyanate					
PRX	20.00	19.65	98.25	0.707	3.60
	50.00	49	98.00	0.707	1.42
	100.00	103.4	103.40	2.40	2.35
CLO	20.00	19.82	99.10	0.127	0.637
	50.00	50.21	100.40	0.148	0.295
	100.00	99.71	99.71	0.205	0.205
II- Using iron(III)-thiocyanate					
PRX	50.00	51.0	102.0	0.707	1.40
	100.00	97.5	97.50	1.76	1.78
	150.00	149.7	99.80	0.212	0.14
CLO	50.00	51.20	102.2	0.848	1.67
	100.00	99.30	99.30	0.494	0.45
	150.00	149.40	99.60	0.459	0.30

Table (2): Analytical parameters for determination of CLO and PRX by the proposed method using Mo (V)-thiocyanate and iron(III)-thiocyanate.

Drug		CLO		PRX	
		using Mo (V)-thiocyanate	using iron(III)-thiocyanate	using Mo (V)-thiocyanate	using iron(III)-thiocyanate
λ_{max} (nm)		470	500	470	500
Conc. Range (µg mL^{-1})		5-120	10-200	10-250	10-200
ε ($l.mol^{-1}.cm^{-1}$)		8.00×10^3	1.70×10^3	9.80×10^3	1.20×10^3
Sandell Sensitivity (µg mL^{-1})		0.125	0.588	0.102	0.833
A= mC +Z	m	0.0087	0.0055	0.0075	0.0098
	z	0	-0.0437	0	-0.0775
SD		0.395-0.919	0.176 – 0.700	0.127 – 1.03	0.254-1.27
RSD		0.396-2.88	0.173- 2.360	0.637- 0.751	0.802- 1.280
LOD (µg mL^{-1})		0.0744	0.109	0.177	0.113
LOQ (µg mL^{-1})		0.223	0.327	0.53	0.340

Table (3): Determination of clomipramine and paroxetine in pharmaceutical preparations.

Drug	Name of Preparation	[Drug] µg mL^{-1} (Taken)	[Drug] (n = 3) Proposed	Official	t-test	F-test
I- Using Mo(V)-thiocyanate						
Clomipramine	Anapramine	20.00 50.00 1000	19.82 50.21 99.71	19.60 49.00 98.00	2.44 11.56 11.79	0. 201 0.044 0.021
Paroxetine	Xanadol	20.00 50.00 100.0	19.00 49.00 103.4	20.20 50.50 101.0	2.04 2.10 1.41	4.00 4.00 11.56
II- Using iron(III)-thiocyanate						
Clomipramine	Anapramine	20.00 50.00 100.0	19.86 51.20 99.30	19.60 49.00 98.00	3.71 3.66 3.71	0.123 1.32 0.122
Paroxetine	Xanadol	20.00 50.00 100.0	20.40 51.00 97.50	20.20 50.50 101.0	1.0 0.4 - 2.8	4.00 4.00 6.25

-Tabulated t–values at 95% confidence limit = 3.182 at degrees of freedom =3
- Tabulated F–values at 95% confidence limit = 9.12.

Reference

1.F. ARIOZ, L. Ersoy, Pharmazie (49), 536 (1995).

2.P. NAGARAJA, M.F. SILWADI, A.A. SYED, Mikrochim. Acta (135), 185 (2000).

3.P. NARAJA, M.F. SILWADI, A.A. SYED, Anal. Lett. 33 (14), 2913 (2000).

4.J. LIMAA, J. PRIOR A, B. REIS B, J. SANTOS A. E. ZAGATTO b. Analytica Chimica Acta (467), 75–81(2002).

5.F. MOHAMED, H. MOHAMED, S. HUSSEIN, S. AHMED. Journal of Pharmaceutical and Biomedical Analysis (39), 139–146 (2005).

6.NAFISUR RAHMAN AND NASHEED AFaq**.** Anal. Methods (2), 513-518 (2010).

7.K. MARQUES, J. SANTOS., J. LIMA. Analytica Chimica Acta (518), 31–36 (2004).

8.T.A. IVANDINI A, B.V. SARADA A, C. TERASHIMA A, T.N. RAO A, D.A. TRYK A, H. ISHIGURO B, Y. KUBOTA B, A. FUJISHIMA A. Journal of Electroanalytical Chemistry (521), 117–126 (2002).

9.J. A. ORTUÑO, J. Hernández and C.Sánchez- Pedreño. Sensors and Actuators B (119), 282–287 (2006).

10.R. THEURILLAT, W. THORMANN, J. Pharm. Biomed. Anal. (18), 751 (1998).

11.H. YOSHIDA, K. HIDAKA, J. ISHIDA, K. YOSHIKUNI, H. NOHTA, M. YAMAGUCHI, Anal. Chim. Acta (413), 137 (2000).

12.C. FRAHNERT, M.L. RAO, K. GRASMAEDER, J. CHROMATOGR. B: ANAL.Technol. Biomed. Life Sci. (794), 35 (2003).

13.R. PIROLA, E. MUNDO, L. BELLODI, S.R. BAREGGI. Journal of Chromatography B (772), 205–210 (2002).

14.H. WEIGMANN, S. HARTTER, C. HIEMKE. Journal of Chromatography B, (710), 227–233 (1998).

15.M.J. BURKE, S.H. PRESKORN. Clin. Pharmacokinet.; 37 (2), 147-165 (1999).

16.J.J. BERZAS-NEVADO, M.J. VILLASENOR-LLERENA, A.M. CONTENTO SALCEDO, E. AGUAS-NUEVO, J. Chromatogr. Sci. (38), 200 (2000).

17.H. KOUA, C. CHENC, Y. HUANGA, W. KOD, H. WUA, S. WUA, Analytica Chimica Acta (525) 23–30 (2004).

18.C.D. AQUILA, J. Pharm. Biomed. Anal. (30), 341–350 (2002).

19.J.P. FOGLIA, D. SORISO, M. KIRSHNER, B.G. POLLOCK, J. Chromatogr. Biomed. Appl.(693), 147 (1997).

20.A. VENKATACHALAM, V. CHATTERJEE.ANALYTICA Chimica Acta (598), 312–317 (2007).

21.MASSAROTI, P. CASSIANO, N. M.DUARTE, L. F. CAMPOS, D. R. MARCHIORETTO, M. A. M. BERNASCONI, G.CALAFATTI, S.BARROS, F. A. P. MEURER, E.C.PEDRAZZOLI, J. J Pharm Pharmaceut Sci . 8(2), 340-347, (2005).

22.C. CALULL, N. DOMINGUEZ .Journal of Chromatography B, (724), 393–398 (1999).

23.A. CHAVES A, S. SILVA B, R. QUEIROZ B, F. LANC¸AS C, M. QUEIROZ A,B. Journal of Chromatography B, (850), 295–302 (2007).

24.M. GROS A, M. PETROVI a,b, D. SCIENCE DIRECT A. Talanta (70), 678–690 (2006).

25.R.N. GUPTA, J. Chromatogr. B, (661), 362 (1994).

26.A. CHAVES, G. JŃIOR, M. QUEIROZ. Journal of Chromatography B, (877), 587–593 (2009).

27.H. NOUWSA, C. MATOS A. A. BARROS B, J. RODRIGUES b. Journal of Pharmaceutical and Biomedical Analysis. (42), 341–346 (2006).

28.M. R. SYED, S. HASHMI, J. B. NAIK**.** International Journal of Pharmacy and Pharmaceutical Sciences. Vol 2, Suppl 2, 0975-1491 (2010).

29.A. ONAL, S.E. KEPEKÇI, A. OZTUNC, J. AOAC Int. (88), 490-495 (2005).

30.I.A. DARWISH, I.H. REFAAT, J. AOAC Int. (89), 326-333 (2006).

31.A.I VOGEL, Quantitative Inorganic Analysis, The Elbs Longmans,London, p. 506 (1986).

32.F.M. ABDEL GAWAD. N.M.ElGuindi, Anal.lett. 28(8), P.1437 (1995).

33.J.Biazek,V.Mres.Chem. Abstr, 22266f (1967).

34.K.N. THIMMAIAH.G.T. GHANDRAPPA, V.C.Sekhar, Mikrochim.Acta (111), 227 (1986).

35.J.C. MILLER and J.N. MILLER, "Statistics for analytical chemistry" 4th ed, Ellis

36.The United States pharmacopeia. The National Formulary usp 26, Rockville, MD (2003).