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ORIGINAL PAPER

Spectrophotometric methods for sertraline hydrochloride and/or clidinium bromide determination in bulk and pharmaceutical preparations

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A spectrophotometric procedure for the determination of sertraline hydrochloride (Sert) and/or clidinium bromide (Clid) in bulk sample and in dosage forms was developed. The purpose of this work was to develop a rapid, simple, inexpensive, precise, and accurate visible spectrophotometric method. The procedure is based on formation of an ion-pair complex by their reaction with bromocresol green (BCG), bromophenol blue (BPB), and bromothymol blue (BTB) in buffered aqueous solution at pH 3. The colored products are extracted into a polar solvent and measured spectrophotometrically at the optimum λ_{max} for each complex. Optimization of different experimental conditions is described. Regression analysis of Beer–Lambert plots showed good correlation in the concentration range of 1–30 μg mL^{-1}. The apparent molar absorptivity, Sandell sensitivity, detection and quantification limits were calculated. For more accurate analysis, Ringbom optimum concentration range of 2–27 μg mL^{-1} was used. The developed methods were successfully applied for the determination of sertraline hydrochloride and clidinium bromide in bulk in pharmaceutical formulations without any interference from common excipients. The procedure has the advantage of being highly sensitive and simple for the determination of the studied drugs, weak UV-absorbing compounds.

Keywords: spectrophotometry, ion pair, sertraline hydrochloride, clidinium bromide, determination, pharmaceutical formulations, urine samples

Introduction

Sertraline hydrochloride (Sert), (1*S*,4*S*)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride, is a selective serotonin reuptake inhibitor (SSRI) with actions and uses similar to those of fluoxetine. Sertraline is slowly absorbed from the gastrointestinal tract with peak plasma concentrations occurring from about 4.5 hours to 8.5 hours after ingestion. It undergoes extensive first-pass metabolism in liver. The main pathway is demethylation to *N*-desmethylsertraline, which is inactive; further metabolism and glucuronide conjugation occurs (Wang et al., 2008). Sertraline is widely distributed throughout body tissues and is highly bound (about 98 %) to plasma proteins.

Two spectrophotometric methods for the determination of sertraline in its dosage forms were described (Meyyanathan et al., 2001). The first method was based on oxidation reaction of sertraline with alkaline potassium permanganate solution; whereas the second method depended on a reaction with 0.1 mass % of 3-methyl-2-benzothiazolinone hydrazone reagent in the presence of 1.0 mass % of ferric chloride solution to give a yellowish green color. UV spectrophotometric determination of sertraline hydrochloride based on direct measurement in pharmaceutical preparations was carried out (Dhake et al., 2000). Also, different spec-

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trophotometric procedures based on ion-pair (Darwish, 2005), charge transfer (Bebawy et al., 1999), and derivative spectrophotometry (Erk, 2003) were used for its determination. Several high performance liquid chromatography (Chen et al., 2004a; Higashi et al., 2005; Venkateswarlu et al., 2007; He et al., 2005), liquid and gas chromatography (Silva et al., 2007; Jain et al., 2005; Gao & Li, 2007; Kim et al., 2002; Chen et al., 2004b) methods have been used for the determination of sertraline hydrochloride.

Clidinium bromide (Clid), (1-methyl-1-azoniabicyclo[2.2.2]oct-8-yl) 2-hydroxy-2,2-diphenyl-acetate bromide, is a quaternary ammonium antimuscarinic with peripheral effects similar to those of atropine. A spectrophotometric method for the determination of clidinium bromide in pharmaceutical formulations containing clidinium bromide and chlordiazepoxide was developed (Özkan et al., 1999). Ratio spectra derivative spectrophotometry was applied to determine clidinium bromide with chlordiazepoxide in their binary mixture. The method was applied to pharmaceutical formulations without any interference from the excipients. Two spectrophotometric methods for the determination of clidinium bromide with chlordiazepoxide in their binary mixture and in its dosage forms were described (Dinç et al., 2006; Toral et al., 1999). A capillary electrophoresis method with indirect UV detection was investigated (Nickerson, 1997). In this method, clidinium bromide (100 mg) was mixed with 1.0 mL of a 1.0 mg mL^{-1} aqueous tetraethylammonium iodide solution (internal standard) and diluted to 10 mL with H_2O. Several high performance liquid chromatography (Oles, 1978) and liquid chromatography methods (Honigberg et al., 1975; Yuen & Lehr, 1991) were described for this determination.

The present paper describes a spectrophotometric procedure that can be applied in laboratories where modern and expensive apparatus such as that required for HPLC, or GLC is not available. The proposed procedure involves the formation of an ion-pair complex between Sert or Clid with BCG, BPB, and BTB as chromophoric reagents. The proposed procedure was successfully applied to determine Sert and Clid with excellent accuracy and precision in pure form, pharmaceutical formulations and in urine samples. The results were compared with those obtained using the official procedures.

Experimental

Instrumentation, materials, and methods

All absorption spectral measurements were made using a Jasco V-530 UV/VIS spectrophotometer (Jasco Inc., Easton, USA), with the scanning speed of 400 nm min^{-1}, and band width of 2.0 nm. Equipped with 10 mm matched quartz cells. pH values of buffer solutions were measured using a HANNA pH meter (HANNA Instruments, Woonsocket, USA), with a calomel electrode as the reference electrode.

All solvents and chemicals used in this study were of analytical grade quality, of the highest purity and used without further purification.

Pure Sert was provided by October Pharma Company for Pharmaceutical Industries (OPC, 6th of October City, Egypt), whereas Clid was kindly supplied by Egyptian International Pharmaceutical Industries Company (EIPICO, 10th of Ramadan City, Egypt. Stock solutions of Sert and Clid (10^{-3} M) were prepared by dissolving 0.03427 g and 0.0432 g of pure Sert and Clid samples, respectively, in the least amount of warm water (50 ± 2 ℃), cooled and transferred to 100 mL measuring flasks, and diluted with water to the mark. Further dilution was carried out with bidistilled water to achieve 100 μg mL^{-1} of the studied drug.

Stock solutions (10^{-3} M) of bromothymol blue (BTB), bromophenol blue (BPB), and bromocresol green (BCG) (Merck, Darmstadt, Germany) were prepared by dissolving an appropriate quantity of the reagent initially in 15 mL of acetone followed by the dilution with acetone to the volume of 100 mL.

Universal buffer solutions of pH 2–12 were prepared as recommended previously (Britton, 1952).

Moodapex tablets, manufactured by OPC, assumed to contain 50 mg of Sert per tablet and Librax tablets, manufactured by EIPICO, claimed to contain 2.5 mg of Clid and 5.0 mg of chlordiazepoxide per tablet were used to determine the studied drugs using the standard addition techniques.

In the molar ratio method, described by Yoe and Jones (1944), concentration of the drug was kept constant (0.5 mL of 10^{-3} M) while that of the reagent was regularly varied (0.1–1.2 mL of 10^{-3} M). Absorbance of the prepared solutions was measured at the optimum wavelength for each complex. The values were then plotted vs. the mole ratio (reagent/drug). Intersection of the obtained straight lines shows the mole ratio of the most stable complexes. A modification of the continuous variation method was utilized to investigate the stoichiometric ratio. A series of solutions was prepared by mixing equimolar solutions of the drug and the reagent in different proportions (0.1–0.9 mL of 10^{-3} M) while keeping the total molar concentration constant (1.0 mL of 10^{-3} M). A plot of the solution absorbance measured at the recommended wavelength versus the mole fraction of the drug showed a maximum at the mole ratio of the most stable complexes.

General procedure

Aliquot portions of Sert and Clid solutions containing 10–300 μg mL^{-1} of the substance were transferred into 10 mL calibrated flasks and mixed with 1.0 mL of the reagent (10^{-3} M) for all ion pairs except for Sert, where BCG and BTB, 0.7 mL and 2.0

mL were used. 2.0 mL of buffer solution of pH 3 using BPB, 3.0 mL and 4.0 mL using BCG and 2.0 mL and 3.0 mL using BTB for Sert and Clid, respectively, were then added. The solution was transferred into a 25 mL separating funnel and the formed ion pair was extracted with 5.0 mL of methylene chloride using BPB for both drugs, BCG and BTB for Clid, and BCG and BTB for Sert; 5.0 mL of carbon tetrachloride and chloroform, respectively, were used and the mixtures were shaken for 3 min. Absorbance of the extracted solutions was measured at 415 nm and 420 nm, using BPB and BCG, and BTB at 410 nm and 416 nm for Sert and Clid, respectively, against extracted blanks prepared similarly, without the drug, after allowed to stand for 3 min. A calibration graph for each drug was constructed; concentration of unknown samples can be deduced by such calibration graph.

Procedure for tablets

The contents of 20 tablets of the investigated drug were thoroughly powdered and mixed and the average weight of each one was determined. An accurately weighed amount of the powder equivalent to 25 mg of Sert or Clid was shaken well with 10 mL of warm water ((50 ± 2)℃) for 5 min, transferred to a 100 mL measuring flask, and filled up to 100 mL with distilled water. Filtration, if necessary, and further dilution with water were carried out to obtain a test solution of 100 μg mL^{-1} of Sert or Clid. The general procedure described above was used for the determination of the drug concentration using the standard addition technique.

Results and discussion

Sertraline hydrochloride and clidinium bromide are amino compounds as they contain secondary and tertiary amines; therefore, attempts were made to determine these substances in aqueous solutions by forming extractable salts or ion pairs between these positively charged compounds in a proper acidic medium and negatively charged indicators as BCG, BPB, and BTB. The theoretical basis of this method is that the dissociation equilibrium of BA-type electrolyte dissociating in aqueous medium according to Eq. (1) (where B^+ is the protonated amino drug moiety and A^- is the BCG, BPB, or BTB anion form) can be shifted to the left (association) if the associate (ion pair) is removed by extraction by means of a solvent immiscible with water.

$$BA \rightleftharpoons B^+ + A^- \quad (1)$$

In order to estimate the optimum experimental conditions facilitating the formation of the ion pair, different parameters can be used.

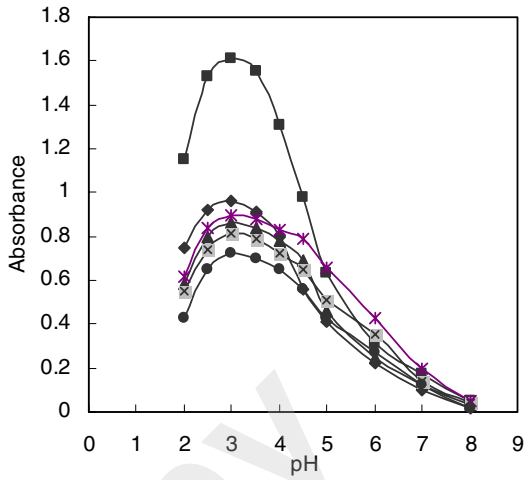

Fig. 1. Effect of various pH values on the absorbance of 10 μg mL^{-1} drug solution with the studied reagents: BCG-Sert (♦), BPB-Sert (■), BTB-Sert (▲), BCG-Clid (×), BPB-Clid (∗), BTB-Clid (●).

Optimization

In order to estimate the optimum pH value for the ion pair complex formation, each drug was allowed to react with the studied reagents in aqueous buffered solution of pH in the range from 2 to 12 (Fig. 1). More intense color was obtained over acidic media of pH 2.5–3.5 using universal buffer solution. Therefore, pH 3 was selected for all further studies. Although the dissociation of BCG and BTB occurs at this pH value the ion pair complex with high color intensity and highest absorbance value is formed. Moreover, the effect of the pH 3 buffer solution on the complex color intensity was studied, indicating that 2.0 mL of buffer solution were sufficient using BPB for both drugs and Sert-BTB ion pairs, whereas 3.0 mL were used for Sert-BCG and Clid-BTB. For the Clid-BCG ion pair, 4.0 mL of the universal buffer solution were needed to achieve the color intensity shown in Fig. 2.

Polarity of the solvent affects both extraction efficiency and absorbance intensity. The results using different extracted solvents (benzene, chloroform, carbon tetrachloride, hexane, and methylene chloride) indicate that methylene chloride is the optimum solvent for all Clid ion pairs; whereas for Sert complexes, carbon tetrachloride, methylene chloride, and chloroform were the best solvents when using BCG, BPB, and BTB, respectively. Those solvents were selected due to their slightly higher sensitivity and considerably lower extraction of the reagent itself. Complete extraction was attained by extraction with 5.0 mL of the used solvent at a time.

The effect of the reagent concentration on the in-

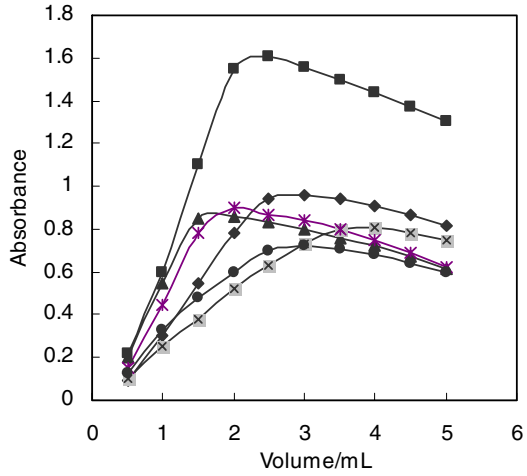

Fig. 2. Effect of buffer (pH 3) volume on the absorbance of 10 μg mL^{-1} drug solution with the studied reagents. For line designation, see legend in Fig. 1.

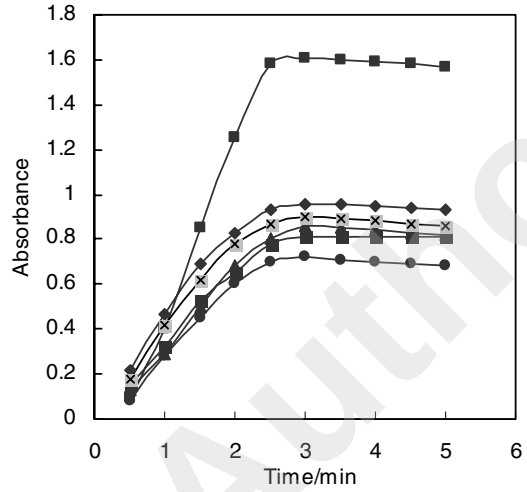

Fig. 3. Effect of shaking time on the absorbance of 10 μg mL^{-1} of the studied drugs with BCG, BPB, and BTB reagents: BCG-Sert (♦), BPB-Sert (■), BTB-Sert (▲), BCG-Clid (■), BPB-Clid (×), BTB-Clid (●).

tensity of color developed at the selected wavelengths was ascertained using different amounts of the reagent (0.4–3.0 mL of 10^{-3} M). For Clid complexes, 1.0 mL of 10^{-3} M was found to be the optimum reagent concentration; whereas for Sert ion pairs, 0.7 mL, 1.0 mL, and 2.0 mL of 10^{-3} M reagents using BCG, BPB, and BTB, respectively, were sufficient for complete color development. Higher concentration of reagents affects the color intensity and the absorbance decreases gradually with the increasing reagent concentration.

The effect of time required for color development completion of ion pair formed between the studied drugs and BCG, BPB, and BTB was investigated. Allowing the reactants to stand and shaking them for different time intervals indicated that 3 min were quite sufficient to obtain maximum color intensity before extraction. During extraction, 3 min were the chosen time of shaking for complete color development (Fig. 3). The formed ion pairs were found to be stable for more than 12 h.

Stoichiometric ratio

Stoichiometry of the ion-pair complex was established by the mole ratio and continuous variation methods. The results show that the ion-pair complex has a 1 : 1 (R : D) ratio and the shape of the resulting curves indicates that the complex is labile. Consequently, a large excess of reagent must be always used to enhance the formation of the complex. The stability constant of the complex was calculated using the data of both methods applying the Harvey and Manning equation and the results are summarized in Table 1.

Quantification

Under the optimum experimental conditions, a linear relationship between absorbance and drug concentration, with a correlation coefficient (r) as given in Table 1, was observed. The regression equations, apparent molar absorptivity calculated from the calibration graph and the Sandell sensitivity were calculated and are presented in Table 1. For more accurate analysis, the Ringbom optimum concentration range was calculated (see Table 1). Standard deviations of the absorbance measurements were calculated from a series of 13 blank solutions. The limits of detection ($K = 3$) and quantification ($K = 10$) of the method were established and are listed in Table 1. According to the IUPAC definition: $C_l = KS_o/s$, where C_l is the limit of detection, S_o is the standard error of blank, s is the slope of the standard curve and K is the constant related to the confidence interval (Inczédy et al., 1998). The relative standard deviations were 0.69 % and 1.14 %, respectively, obtained from a series of 10 standards each containing 10 μg mL^{-1} of the drug.

In order to determine the accuracy and precision of the proposed methods, solutions containing five different concentrations of Sert or Clid were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 2.

Interference

A systematic quantitative study was carried out by measuring the absorbance of solutions containing 10 μg mL^{-1} of Sert or Clid with varying excess of excipients, additives, and degradation products using the recommended methods. No significant interference was observed from the commonly used additives and excipients, such as glucose, lactose, fructose, calcium hydrogen phosphate, magnesium stearate, and starch,

Table 1. Analytical characteristics of the proposed procedures

Parameter[a,b]	Sert			Clid		
	BCG	BPB	BTB	BCG	BPB	BTB
pH	3	3	3	3	3	3
λ_{max}/nm	415	415	410	420	420	416
Extracting solvent	CCl_4	CH_2Cl_2	$CHCl_3$	CH_2Cl_2	CH_2Cl_2	CH_2Cl_2
Stability constant	3.96	4.35	4.45	4.47	4.72	4.29
Beer's concentration range/(μg mL^{-1})	2.0–18	1.0–12	3.0–22.5	2.5–20	2.5–20	4.0–30
Ringbom optimum range/(μg mL^{-1})	3.0–15	2.0–10	4.0–20	3.0–18.5	3.0–18	5.0–27
DL/(μg mL^{-1})	0.55	0.28	0.97	0.75	0.81	1.23
QL/(μg mL^{-1})	1.9	0.97	2.83	2.95	2.40	3.98
Molar absorptivity/(L mol^{-1} cm^{-1})	3.29×10^4	5.52×10^4	2.95×10^4	3.49×10^4	3.89×10^4	3.13×10^4
Sandell sensitivity/(ng cm^{-2})	10.4	6.2	11.6	12.4	11.1	13.8
Slope	0.096	0.161	0.086	0.081	0.09	0.072
RSD of slope/%	0.0083	0.0049	0.0072	0.0072	0.0068	0.0053
Intercept	−0.012	−0.0058	−0.0175	−0.0060	−0.0084	−0.0132
Correlation coefficient	0.9993	0.9996	0.9996	0.9992	0.9996	0.9998
RSD/%	0.75	0.93	1.13	1.14	0.69	0.94
RE/%	±1.50	±1.20	±1.65	±1.40	±1.25	±1.35
Calculated t-values[c]	1.16	1.36	1.57	1.38	1.32	1.17
Calculated F-test[c]	2.57	2.83	3.18	2.32	2.67	2.49

a) Regression equation: $A = a + bC$, where C is the concentration in μg mL^{-1}; *b*) DL means detection limits; QL means quantification limits; RSD is the relative standard deviation; RE is the range of error; *c*) theoretical values for t-value and F-test at the 95 % confidence level and 5 degrees of freedom are 2.57 and 5.05, respectively.

Table 2. Evaluation of accuracy and precision of the proposed methods[a]

Sert ion-pair	Taken (μg mL^{-1})	Found[b] (μg mL^{-1})	Recovery (%)	RSD (%)	RE (%)	CL[c]	Clid ion-pair	Taken (μg mL^{-1})	Found[b] (μg mL^{-1})	Recovery (%)	RSD (%)	RE (%)	CL[c]
BTB	2.0	2.02	101.00	0.94	0.99	2.02 ± 0.020	BTB	2.5	2.47	98.80	0.65	0.68	2.47 ± 0.017
	4.0	3.98	99.50	0.68	0.72	3.98 ± 0.029		5.0	5.01	100.20	0.76	0.80	5.01 ± 0.040
	6.0	6.03	100.50	1.08	1.13	6.03 ± 0.068		7.5	7.56	100.80	0.57	0.60	7.56 ± 0.045
	12.0	11.92	99.33	1.17	1.24	11.92 ± 0.049		10.0	10.06	100.60	1.10	1.16	1.06 ± 0.047
	18.0	17.85	99.17	0.94	1.22	17.85 ± 0.029		15.0	15.06	100.40	0.89	0.94	15.06 ± 0.029
BPB	2.0	2.03	101.50	0.45	0.47	2.03 ± 0.0095	BPB	2.5	2.51	100.40	0.64	0.67	2.51 ± 0.017
	4.0	3.95	98.75	0.68	0.72	3.95 ± 0.083		5.0	4.98	99.60	0.56	0.59	4.98 ± 0.029
	6.0	5.97	99.50	0.59	0.62	5.97 ± 0.037		7.5	7.55	100.67	0.62	0.65	7.55 ± 0.049
	12.0	12.05	100.42	1.13	1.23	12.05 ± 0.053		10.0	10.05	100.50	0.83	0.98	10.05 ± 0.044
	15.0	14.90	99.33	0.94	0.99	14.90 ± 0.049		12.5	12.42	99.36	0.87	0.97	12.42 ± 0.067
BCG	2.0	1.97	98.50	1.17	1.22	1.97 ± 0.024	BCG	2.5	2.497	99.88	1.04	1.09	2.497 ± 0.027
	4.0	4.01	100.25	0.92	0.97	4.01 ± 0.039		5.0	5.03	100.60	0.95	1.00	5.03 ± 0.050
	6.0	6.04	100.67	0.91	0.96	6.04 ± 0.058		7.5	7.47	99.60	0.90	0.94	7.47 ± 0.071
	11.0	11.05	100.45	0.78	0.84	11.05 ± 0.079		10.0	9.97	99.70	0.98	1.04	9.97 ± 0.063
	16.0	15.90	99.38	1.04	1.12	15.90 ± 0.058		15.0	14.92	99.46	0.69	0.73	14.92 ± 0.056

a) For abbreviations see Table 1; *b*) average of six determinations; *c*) 95 % confidence level and five degrees of freedom.

for the examined methods. Also, there was no interference from common degradation products (results of thermal and hydrolytic treatment) which are likely to occur under normal storage condition.

Analytical applications

The proposed method was successfully applied for the determination of Sert and Clid in pharmaceutical formulations using the standard addition method. The obtained results, given in Table 3, are in good agreement with those obtained by the official methods based on potentiometric titration in mercuric acetate with 0.1 M perchloric acid using crystal violet as indicator (to bluish green end point) for Sert (October Pharma Company, 2005) and on potentiometric titration with 0.1 M perchloric acid in dioxane for Clid (United States Pharmacopoeial Convention, 2002). The results obtained were compared statistically (Miller & Miller, 2000) by the Student's t-value (for accuracy) and variance ratio F-test (for precision) with the official methods (October Pharma Company, 2005; United States Pharmacopoeial Convention, 2002) at the 95 % confidence level, as recorded in Table 3. The results showed that the t- and F-values were lower than the critical values with high

Table 3. Determination of Sert and Clid in pharmaceutical formulations applying the standard addition technique

Pharmaceutical preparation	Taken ($\mu g\ mL^{-1}$)	Added ($\mu g\ mL^{-1}$)	Found[a] ($\mu g\ mL^{-1}$)				t-value[b]			F-test[b]		
			BPB	BCG	BTB	Official	BPB	BCG	BTB	BPB	BCG	BTB
Librax[c]	3.0	–	3.02	2.98	2.97	2.93	1.43	1.54	1.36	2.98	3.52	2.89
	3.0	2.0	5.04	5.03	4.98	4.83	1.21	1.24	1.43	2.61	3.07	3.28
	3.0	4.0	6.98	7.05	7.02	7.15	1.39	1.72	1.57	3.25	2.89	3.44
	3.0	6.0	8.95	9.06	8.96	8.88	1.34	1.11	1.21	2.87	2.36	3.17
	3.0	8.0	11.07	11.05	10.97	11.18	1.80	1.31	1.24	3.17	2.56	3.23
Moodapex[d]	3.0	–	2.98	3.02	2.97	3.05	1.76	1.46	1.87	3.09	3.12	2.98
	3.0	2.0	5.02	5.04	5.02	4.92	1.54	1.81	1.64	3.22	3.05	3.46
	3.0	4.0	6.97	6.95	7.03	7.10	1.81	1.73	1.59	2.98	3.59	3.53
	3.0	6.0	9.03	8.97	8.95	9.15	1.68	1.57	1.91	3.09	2.97	4.10
	3.0	8.0	10.99	11.03	10.94	10.85	1.89	1.93	1.48	2.78	2.47	2.41

a) Average of six determinations; *b*) theoretical values for t-value and F-test at the 95 % confidence level and 5 degrees of freedom are 2.57 and 5.05, respectively; *c*) 2.5 mg per tablet; *d*) 50 mg per tablet.

percentage recoveries indicating that no interference from additives and excipients on the formulations was found. Consequently, the methods represent a simple, rapid, accurate, and stability indicating assay. Therefore, the proposed methods can be recommended for routine analysis of Sert and Clid in pure as well as in dosage forms in the majority of drug quality control laboratories.

Conclusions

The proposed methods are simpler, less time consuming and more sensitive than the official methods based on potentiometric titration (October Pharma Company, 2005; United States Pharmacopoeial Convention, 2002). All proposed procedures were advantageous over other reported methods with respect to their higher sensitivity allowing the substance determination of up to 1.0 $\mu g\ mL^{-1}$, simplicity, reproducibility, precision, accuracy, and stability of the colored species. The results obtained were compared statistically by the Student's t-value (for accuracy) and variance ratio F-test (for precision) with the official methods (October Pharma Company, 2005; United States Pharmacopoeial Convention, 2002) at the 95 % confidence level. The proposed methods can be applied for routine analysis and in quality control laboratories for the quantitative determination of Sert and Clid in bulk and in pharmaceutical formulations without interference from excipients and additives.

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