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Validated Spectrophotometric Methods for Determination of Selegiline HCl as an Irreversible Inhibitor of Monoamine Oxidase in Pure Form and Pharmaceutical Formulations

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Abstract
Sensitive, simple, precise, reproducible, and validated spectrophotometric methods have been developed for the determination of an irreversible inhibitor of monoamine oxidase (selegiline hydrochloride) in pure form and pharmaceutical formulations. The methods are based on the formation of a yellow-colored ion-pair complex between selegiline hydrochloride and three acid dyes, namely, bromocresol green (BCG), bromothymol blue (BTB), bromophenol blue (BPB), and bromocresol purple (BCP), in an acidic buffer solution with absorption maxima at 420, 415, 414, and 410 nm, respectively. Several parameters, such as pH, buffer type and volume, reagent volume, sequence of addition, and effect of extracting solvent, were optimised. Under the optimum experimental conditions, Beer's law is obeyed over the concentration ranges of 1.0–14, 1.0–12, 1.0–10, and 1.0–16 µg/ml for BCG, BTB, BPB, and BCP, respectively, with good correlation coefficients (0.9992-0.9998). The apparent molar absorptivity's and Sandell's sensitivity values are reported for all methods. The limit of detection (LOD) and the limit of quantification (LOQ) values are found to be 0.27, 0.3, 0.29, and 0.3 µg/ml and 0.90, 1.0, 0.97, and 1.0 µg/ml for BCG, BTB, BPB, and BCP, respectively. The stoichiometric ratio of the formed ion-pair complexes was found to be 1:1 (drug: reagent) for all methods, as deduced by Job's method of continuous variation. The proposed methods were successfully applied for the determination of selegiline hydrochloride in pharmaceutical formulations with good accuracy and precision. A statistical comparison of the results was performed using the Student's t-test and variance ratio F-test at the 95% confidence level, and there was no significant difference between the reported and proposed methods regarding accuracy and precision. Further, the validity of the proposed methods was confirmed by recovery studies via standard addition techniques in accordance with ICH guidelines.

Keywords
Selegiline hydrochloride, Ion-pair complex, Acid dyes, Spectrophotometry, Method validation, Pharmaceutical formulations

INTRODUCTION

Selegiline hydrochloride (SLG) is chemically designated as (R)-N-methyl-N-(1-phenylpropan-2-yl)prop-1-yn-3-amine (Figure 1) [1]. It is a selective, irreversible inhibitor of monoamine oxidase (MAO-A) used for the treatment of earlystage Parkinson's disease, depression, and senile dementia [2]. It is useful in veterinary medicine to treat the symptoms of Cushing's disease and cognitive dysfunction (canine cognitive dysfunction) in dogs [3]. Further, increase in the dosage of selegiline more than 10 mg/day may lead to the non-selective inhibition of MAO [4]. Hence, it is necessary to develop a method for the determination of SLG in pure form and in pharmaceutical formulations.

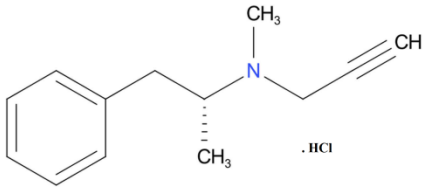


Fig. 1 The chemical structure of selegiline hydrochloride (SLG)

The literature survey reveals that very few methods were reported for the estimation SLG in pharmaceutical formulations which include high performance liquid chromatography [5–13], gas chromatography [14, 15], fluorescence polarization immunoassay (FPIA) and gas chromatography-mass spectrometry (GC/MS) [16], spectrofluorometry [17], stereoselective analyses [18], capillary electrophoresis [19, 20] and A flow-injection chemiluminescence [21], potentiometry [22]. Spectrophotometric method [23, 24] were reported but most of these methods are either not appropriately sensitive or tedious and utilized expensive instruments that are not available in most quality control laboratories. For these reasons, it was worthwhile to develop a new, simple, cost effective and selective spectrophotometric method for the determination of SLG in its pharmaceutical dosage forms.

Visible spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories, hospitals and pharmaceutical industries for the assay of different classes of drugs in pure form, pharmaceutical formulations and biological samples, due to its simplicity, less expensive, less time consuming and reasonable sensitivity with significant economic advantages.

The aim of the present work is to develop simple, sensitive, accurate, precise, low-cost and validated extractive spectrophotometric methods for the determination of SLG in pure form and pharmaceutical formulations. The proposed methods are based on the ability of SLG to form stable ion-pair complexes with bromocresol green (BCG), bromothymol blue (BTB), bromophenol blue (BPB), and bromocresol purple (BCP) in acidic buffer solution. No interference was observed in the assay of SLG from common excipients in levels found in dosage forms. These methods are validated by statistical data.

MATERIAL AND METHODS

Instrumentation

All absorption spectra were made using a Shimadzu UV-1601 UV/visible double beam spectrophotometer (Sweden) equipped with 10 mm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm. The pH values of different buffer solutions were checked using a Hanna pH-meter instrument (pH 211) (Romania) equipped with a combined glass-calomel electrode.

Materials and Reagents

All reagents, chemicals and solvents used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the investigation.

Pure sample of SLG was kindly supplied by Eva Pharma, Egypt, with a purity of 99.70±1.0% by applying the reported method [23]. Parkineast tablets, labeled to contain 10 mg SLG per tablet, a product of Western Pharmaceutical Industries Company, Cairo, Egypt. Tremogine tablets, labeled to contain 10 mg SLG per tablet, a product of Eva Pharma, Egypt were purchased from local pharmacies.

Preparation of stock standard solution

Stock standard solutions (100 µg/ml) and (1.0×10^{-3} mol/l) of SLG were prepared by dissolving 10 and 22.37 mg of pure SLG in bidistilled water and diluted to the mark in a 100 ml volumetric flask. The standard solutions were stable for at least 7.0 days when kept in the refrigerator. Serial dilution with the same solvent was performed to obtain the appropriate concentration range

Reagents

BPB, BCG, BTB, BCP, and MO (BDH Chemicals LTD, Poole, England) and used without further purification. Stock solutions (0.1%, w/v) or (1.0×10^{-3} mol/l) of reagents were prepared by dissolving the appropriate weight of each reagent in10 ml of 96% ethanol and diluted to 100 ml with bidistilled water. These solutions were kept in the refrigerator.

Series of buffer solutions of NaOAc–HCl (pH=1.99-4.92), NaOAc–AcOH (pH=3.4-5.6) and potassium hydrogen phthalate–HCl (pH=2.0-7.0) were prepared by following the standard methods [25]. The pH of each solution was adjusted

to an appropriate value by the addition of 0.2 mol/l hydrochloric acid or sodium hydroxide with the help of the pH meter. Freshly prepared solutions were always employed. Chloroform, methylene chloride, and carbon tetrachloride were obtained from (BDH Chemicals Ltd., Poole, England) and anhydrous sodium sulfate was obtained from (Prolabo).

General recommended procedure

Accurately measured aliquots (0.1–1.6 ml) of standard SLG solution (100 µg/ml) was transferred into 10 ml measuring flasks. 3.0 ml NaOAc– AcOH buffer at the optimum pH 4.0, 3.0, 4.5 and 3.0 using BCG, BTB, BPB and BCP, respectively were added. Then, 2.0 ml of each reagent (0.1%, w/v) was added and the volume was completed to 10 ml with bidistilled water. The formed ion associate complexes were extracted with 10 ml methylene chloride. The solution was shaking for 2.0 min, then allowed to stand for clear separation of the two phases and the methylene chloride layer was passed through anhydrous sodium sulfate. The absorbance of the yellow colored ion-pair complexes was measured at 420, 415, 414 and 410 nm using BCG, BTB, BPB and BCP, respectively against corresponding reagent blank similarly prepared. All measurements were made at room temperature. In the three proposed methods, a standard curve was prepared by plotting the absorbance values versus concentrations of SLG to calculate the amount of drug in unknown analyte samples.

Applications for dosage forms

Twenty tablets containing SLG were finely pulverized and weighed. A weighed quantity of the powdered tablets equivalent to 10 mg of SLG was transferred into a 100 ml volumetric flask, about 20 ml of bidistilled water was added and the flask was sonicated for 30 min. The volume was completed to the mark with bidistilled water, mixed well and filtered through a Whatman No.1 filter paper into 100 ml volumetric flask, discarding the first 10 ml, then the conical flask was washed with bidistilled water. The wash was added to the same volumetric flask, and then the flask was made up to volume with bidistilled water. Aliquots containing SLG in the final concentration ranges were analyzed as described under "General recommended procedure". The concentration of SLG was determined either from the calibration curve or using the corresponding regression equation. The method of standard addition was used for the accurate determination of SLG content.

Stoichiometric relationship

The stoichiometric ratios of the ion-associates formed between SLG and the reagents were determined by applying the continuous variation [26] and the molar ratio [27] methods at the optimum wavelengths. In continuous variation method, equimolar solutions were employed: a 1.0 x 10^{-4} mol/l standard solution of SLG and 1.0 x 10^{-4} mol/l solution of dye were used. A series of solutions was prepared in which the total volume of SLG and the dye was kept at 2.0 ml. The drug and reagent were mixed in various complementary proportions (0.2:1.8, 0.4:1.6, 0.6:1.4, 0.8:1.2, 1.0:1.0, 1.2:0.8, 1.4:0.6, 1.6:0.4, 1.8:0.2) and completed to volume in a 10 mL calibrated flask with the appropriate solvent for extraction following the above mentioned procedure. In the molar ratio method, the concentration of SLG is kept constant to 1.0 ml of (1.0 x10^{-4} mol/l) while that of dye (1.0 x10^{-4} mol/l) is regularly varied (0.2–2.4 ml). The absorbance of the prepared solutions was measured at optimum condition and at the optimum wavelength for each complex.

RESULTS AND DISCUSSION

Absorption Spectra

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes present mainly in anionic form at pH ≥ 2.5. So, when treated with an acid dye at acidic pH using buffer solutions, a yellow ion-pair complex which is extracted with methylene chloride is formed. The absorption spectra of the ion-pair complexes, which were formed between SLG and reagents were measured in the range 350–550 nm against the blank solution and the maximum absorbances were measured at wavelengths 420, 415, 414 and 410 nm using BCG, BTB, BPB and BCP, respectively (Figures 2 and 3).

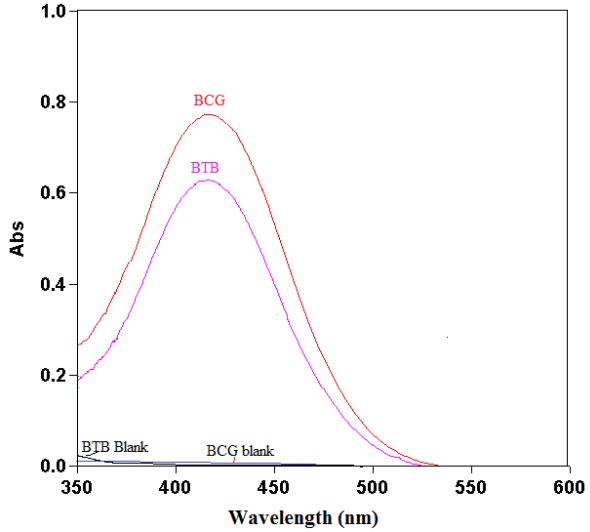


Fig. 2 Absorption spectra of ion-pair complexes of 14 and 12 µg/ml SLG using (1.0 x 10^{-3} mol L^{-1}) BCG and BTB reagents, respectively against reagent blank

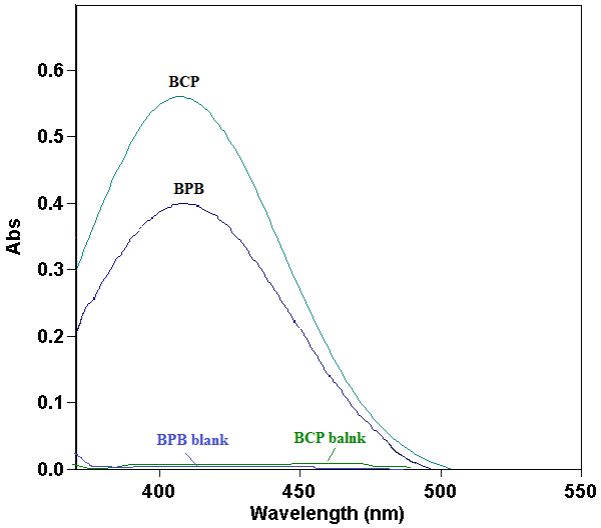


Fig. 3 Absorption spectra of ion-pair complexes of 10 and 16 µg/ml SLG using (1.0 x 10^{-3} mol L^{-1}) BPB and BCP reagents, respectively against reagent blank

Optimum reaction conditions for complex formation

The optimization of the methods was carefully studied to achieve the complete reaction formation, highest sensitivity and maximum absorbance. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as pH of buffer, the type of organic solvent, volumes of the dye, reaction time and temperature for the extraction of ion-pair complexes.

Effects of pH

It was observed that the effective extraction of the complex depends on the type of the buffer used and its pH value. The effect of pH was studied by extracting the colored complexes in the presence of various buffers such as NaOAc–HCl (pH=2.0-4.5), NaOAc–AcOH (pH=2.8-5.5) and potassium hydrogen phthalate–HCl (pH=3.0-6.0). It is evident that the maximum color intensity and maximum absorbance were found in NaOAc-AcOH buffer. It is evident that the maximum absorbances of the ion pair complexes were obtained at pH 4.0, 3.0, 4.5, and 3.0 using BCG, BTB, BPB and BCP, respectively (Figure 4). Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5-5.0 ml). The higher absorbance value and reproducible results were obtained by using 3.0 ml of acetate buffer solutions.

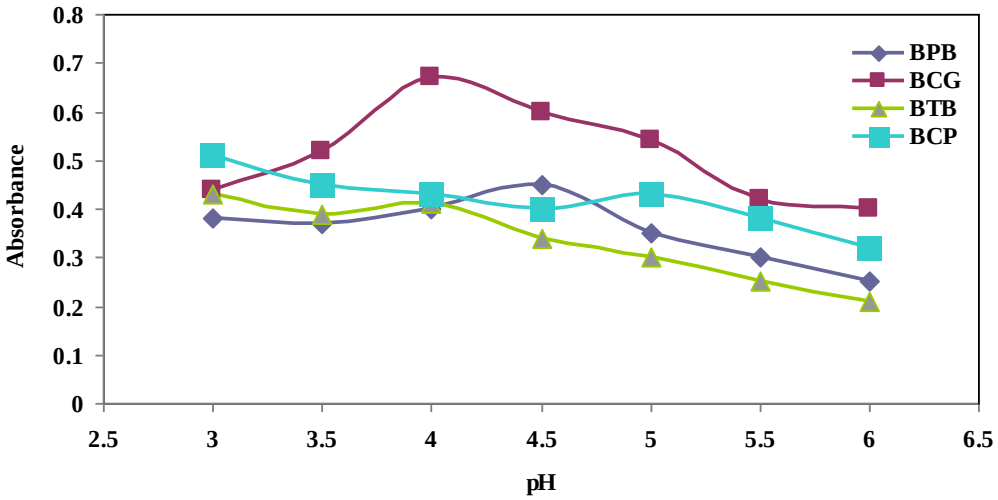


Fig. 4 Effect of pH of buffer solution on the ion pair complex formation between (14, 12, 10 and 16 µg/ml) SLG and (0.1%, w/v) BCG, BTB, BPB and BCP reagents, respectively, (N=3.0)

Effect of reagent concentration

The effect of the reagent was studied by measuring the absorbance's of solutions containing a fixed concentration of SLG and various volumes of the BCG, BTB, BPB and BCP (0.1%, w/v) reagents in the range of (0.5–4.0 ml). The results showed that the absorbance of the extracted ion-pair increased by increasing the reagent volume till 2.0 ml. So, the maximum color intensity of the complex was achieved with 2.0 ml of (0.1 %, w/v) of each reagent solution. Although a larger volume of the reagent had no pronounced effect on the absorbance's of the formed ion-pair complexes (Figure 5).

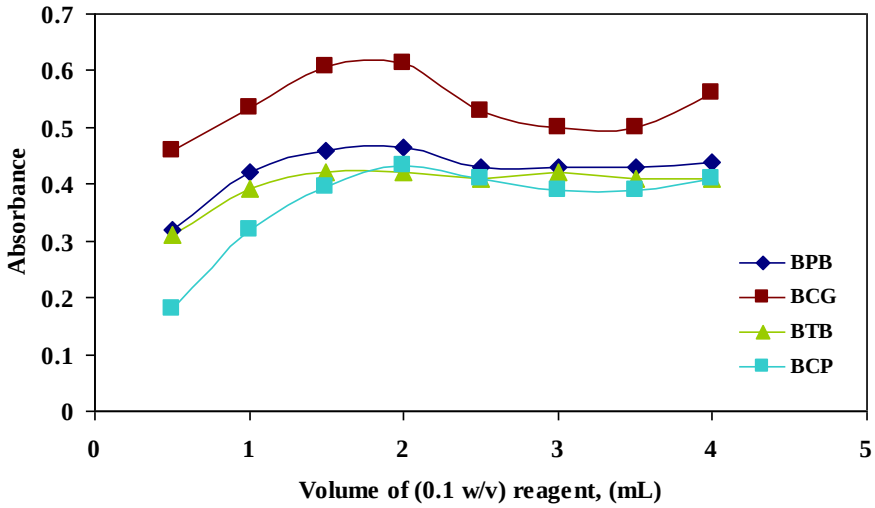


Fig. 5 Effect of volume of (0.1%, w/v) BCG, BTB, BPB and BCP reagent on the ion pair complex formation with (14, 12, 10 and 16 µg/ml) SLG, (N=3.0)

Choice of extracting solvent

The effect of several organic solvents *viz*., chloroform, carbon tetrachloride, dichloromethane and diethylether were studied for effective extraction of the colored species from the aqueous phase (Figure 6). Dichloromethane was found to be the most suitable solvent for extraction of colored ion pair complexes for all reagents quantitatively. Experimental results indicated that double extraction with total volume 10 ml dichloromethane, yielding maximum absorbance intensity, stable absorbance and considerably lower extraction ability for the reagent blank and the shortest time to reach the equilibrium between both phases.

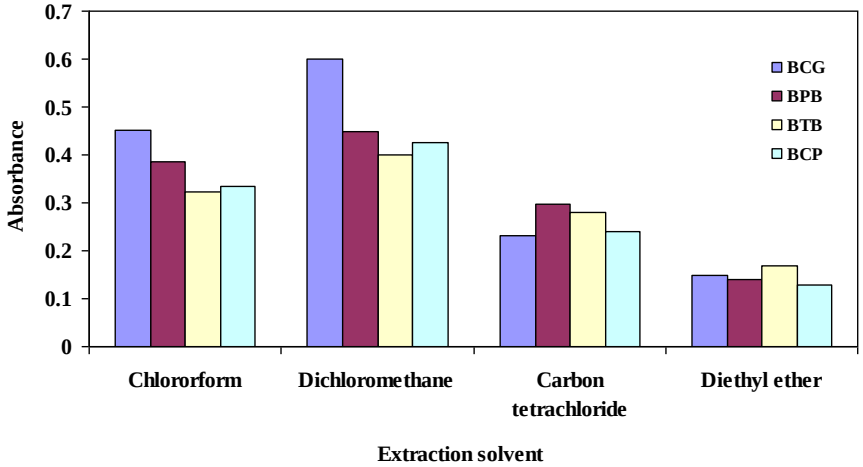


Fig. 6 Effect of extraction solvent on the ion pair complex formation of SLG with dyes at the optimum conditions

Effect of shaking time and temperature

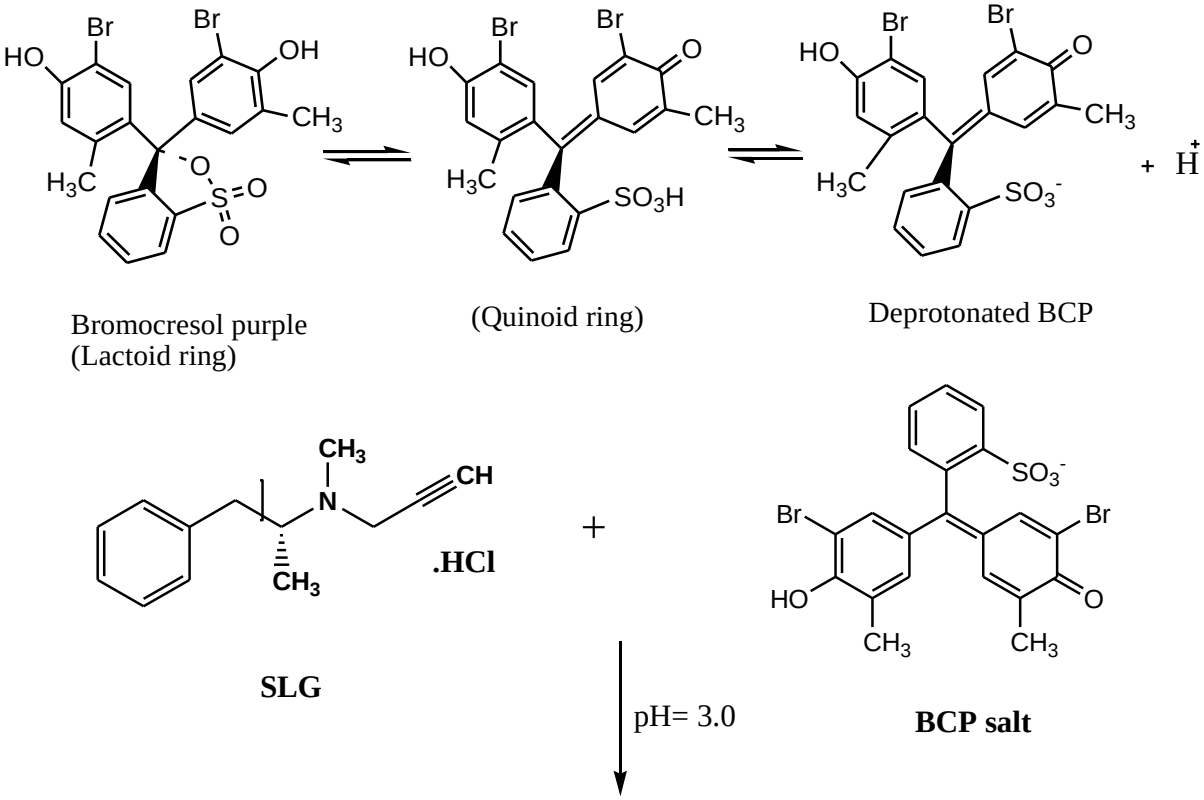
The optimum shaking time was investigated by shaking from 0.5-5.0 min at ambient temperature (25 ± 2°C). Maximum and constant absorbance value were obtained when extracted after 2.0 min of shaking for all complexes. Therefore, shaking time of 2.0 min was maintained throughout the experiment. The effect of temperature on colored complexes was studied by measuring the absorbance values over the temperature range 20-35°C. It was found that the absorbance of the colored ion pair complex was constantly up to 30°C. At higher temperatures, the drug concentration was found to increase due to the volatile nature of dichloromethane. Therefore, the temperature chosen was room temperature (25 ± 2°C) as the best temperature for determination of SLG in bulk and pharmaceutical formulations. The absorbance of the complexes remains stable for at least 12 h at room temperature.

Composition of the ion-pair complexes

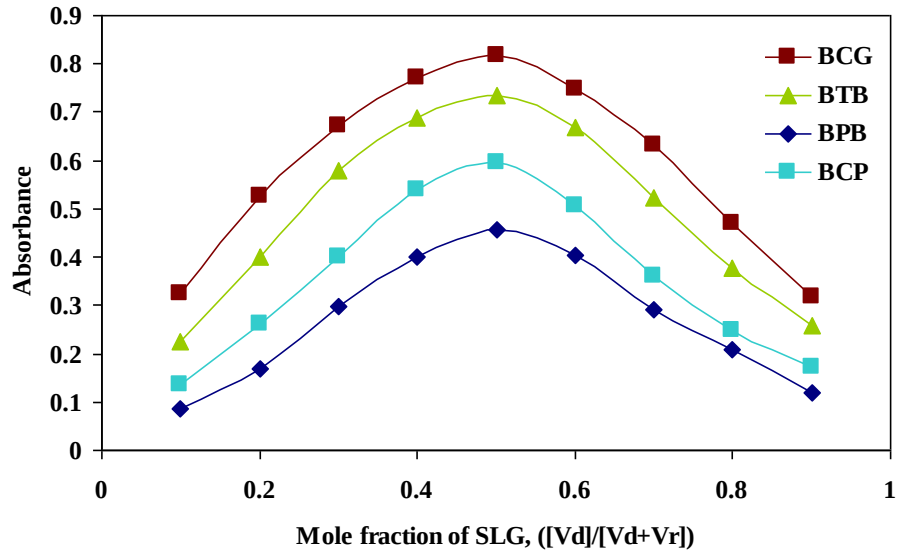
The molar ratio of the ion pair complexes (SLG: dye) was determined by the continuous variations and molar ratio methods (Figures 7 and 8). The results indicate that the molar ratio of (SLG: dye) is (1:1) ion-pair complex are formed through the electrostatic attraction between the positive charged SLG^+ and negatively charged dye, (BCG^-, BTB^-, BPB^-, and BCP^-). The extraction equilibrium can be represented as follows:

$$SLG^+_{(aq)} + D^-_{(aq)} \longleftrightarrow SLG^+D^-_{(aq)} \longleftrightarrow SLG^+D^-_{(org)}$$

where SLG^+ and D^- represent the protonated drug and the anion of the dye (BCG^-, BTB^-, BPB^-, and BCP^-), respectively, and the subscript (aq) and (org) refer to the aqueous and organic phases, respectively (Scheme 1).



Scheme 1 Proposed reaction mechanism for the ion pair complex formation between SLG and BCP



1.

Fig. 7 Job's method of continuous variation graph for the reaction of SLG with BCG, BTB, BPB and BCP, [drug] = [dye] = (1.0×10^{-4} mol/l) (N=3.0)

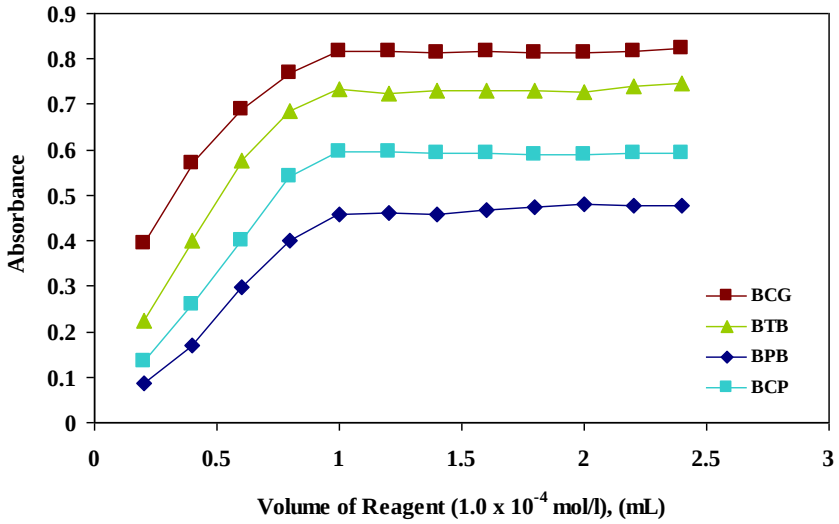


Fig. 8 Mole ratio plots for the ion-association complexes of SLG (1.0 x 10^{-4} mol/l) with various volumes of reagent solution (1.0 x 10^{-4} mol/l) at the optimum conditions

Method of Validation

Linearity

At described experimental conditions for SLG determination, standard calibration curves with reagents were constructed by plotting absorbance vs. concentration of SLG. The statistical parameters were given in the regression equations calculated from the calibration graphs A = aC + b, where A is the absorbance and C is the concentration in µg/ml. The linearity of calibration graphs was proved by the high values of the correlation coefficient (*r*) and the small values of the *y*-intercepts of the regression equations. The apparent molar absorptivity of the resulting colored ion-pair complexes and relative standard deviation of response factors for each proposed spectrophotometric method were also calculated and recorded in Table 1. The molar absorptivity of BCG > BTB > BCP > BPB ion-pair complexes.

Sensitivity

The limits of detection (LOD) and quantitation (LOQ) for the proposed methods were calculated using the following equation [28, 29]:

$$LOD = 3s / k \text{ and}$$
$$LOQ = 10\ s / k$$

where *s* is the standard deviation of ten replicate determinations values of the reagent blank and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, LOD and LOQ were found to be 0.27, 0.3, 0.29, and 0.3 µg/ml and 0.90, 1.0, 0.97 and 1.0 µg/ml for BCG, BTB, BPB, and BCP, respectively.

Table 1 Statistical analysis of calibration graphs and analytical data in the determination of SLG using the proposed methods

Parameters	BCG	BTB	BPB	BCP
Wavelengths λ_{max} (nm)	420	415	414	410
Beer's law limits (µg/ml)	1.0-14	1.0-12	1.0-10	1.0-16
Molar absorptivity ε, (l mol^{-1} cm^{-1}) x 10^4	1.832	1.724	1.309	1.585
Sandell's sensitivity (ng cm^{-2})	12.21	12.98	17.09	14.12
Regression equation [a]				
Intercept (*a*)	0.0091	0.0032	- 0.0006	0.0056
Standard deviation of intercept (*Sa*)	0.009	0.007	0.008	0.007
Slope (b)	0.0542	0.0546	0.0401	0.037
Standard deviation of slope (*Sb*)	0.01	0.008	0.006	0.009
Correlation coefficient (*r*)	0.9994	0.9997	0.9996	0.9998
LOD (µg/ml) [b]	0.27	0.30	0.29	0.30
LOQ (µg/ml) [b]	0.90	1.0	0.97	1.0
Mean ± SD	99.60 ± 0.65	99.90 ± 0.84	100.10 ±0.75	99.50 ± 0.90
RSD%	0.65	0.84	0.75	0.90
RE%	0.68	0.88	0.79	0.94
t-test [c]	0.28	0.45	0.70	0.70
F- test [c]	1.25	1.38	1.51	1.10

[a] A = a + bC, where C is the concentration in µg/ml, A is the absorbance units.

[b] LOD, limit of detection; LOQ, limit of quantification; SD , standard deviation; SE , standard error; RSD%, relative standard deviation; RE%, relative error.

[c] The theoretical values of t and F at P= 0.05 are 2.57 and 5.05, respectively.

Accuracy and precision

In order to evaluate the accuracy and precision of the proposed methods, solutions containing three different concentrations of SLG were prepared and the assay procedure was analyzed in six replicates, and percentage relative standard deviation (RSD%) values were obtained within the same day to evaluate the repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision). The percentage relative error (RE%) was calculated using the following equation:

$$RE\ \% = [(Founded - Added) / Added] \times 100$$

The analytical results of intra-day and inter-day precision (RSD%) and accuracy (RE%) were summarized in Tables 2. These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

Robustness and Ruggedness

For the evaluation of the method robustness, some parameters were interchanged; pH, dye concentration, wavelength range, and shaking time. The capacity remains unaffected by small deliberate variations. Method ruggedness was expressed as RSD% of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical differences between different analysts and instruments, suggesting that the developed methods were robust and rugged (Table 3).

Effects of interference

To assess the usefulness of the method, the effect of diluents, excipients and additives which often accompany SLG in its dosage forms (starch, lactose, glucose, saccharose, talc, sodium chloride, titanium dioxide, and magnesium stearate) was studied. The results indicated that there is no interference from excipients and additives, indicating a high selectivity for determining SLG in its dosage forms.

Applications to dosage forms

The proposed methods have been successfully applied to the determination of SLG in dosage forms. Six replicates determinations were made. Moreover, to check the validity of the proposed methods, dosage forms were tested for possible interference with standard addition method (Table 4). Therefore, it is concluded that the excipients in dosage forms of SLG did not cause any interference in the analysis of SLG. A statistical comparison of the results for determination of SLG in tablet dosage forms using the proposed and reported methods [23] is shown in Table 5. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed and reported methods at the 95 % confidence level with respect to accuracy and precision [29] (Table 5).

Table 2 Intra-day and Inter-day precision and accuracy data for SLG obtained by the proposed methods

Method	Added concentration (µg/ml)	Intra-day				Inter-day			
		Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]
BCG	4.0	99.30	0.78	-0.70	3.972± 0.031	99.70	0.65	-0.30	3.988 ± 0.026
	8.0	99.40	1.25	-0.60	7.952± 0.099	99.00	0.90	1.00	7.92 ± 0.071
	12	99.80	1.80	-0.20	11.952± 0.215	99.10	1.30	-0.90	11.892 ± 0.155
BTB	4.0	99.50	0.57	-0.50	3.980 ± 0.023	99.40	0.69	-0.60	3.976 ± 0.027
	6.0	99.70	0.85	-0.30	5.982 ± 0.051	99.80	0.87	-0.20	5.988 ± 0.052
	8.0	100.40	1.12	0.40	8.032 ± 0.09	99.20	1.24	-0.80	7.936 ± 0.098
BPB	4.0	100.10	0.71	0.10	4.004 ± 0.028	99.30	0.52	-0.70	3.972 ± 0.021
	8.0	99.30	1.14	-0.70	7.944 ± 0.091	99.50	0.76	-0.50	7.960± 0.637
	16	99.80	1.46	-0.20	15.968 ± 0.233	100.20	0.97	0.20	16.032± 0.156
BCP	4.0	99.60	0.63	-0.40	3.984 ± 0.025	100.30	0.80	0.30	4.012 ± 0.032
	8.0	99.90	0.92	-0.10	7.992 ± 0.074	99.40	1.19	-0.60	7.952 ± 0.095
	12	99.10	1.40	-0.90	11.892 ± 0.166	100.00	1.63	0.00	12.0 ± 0.196

[a] Mean of six determination, RSD%, percentage relative standard deviation; RE%, percentage relative error.
[b] Confidence limit at 95% confidence level and five degrees of freedom (t = 2.571).

Table 3 Results of method robustness and ruggedness expressed as intermediate precision (RSD%) for SLG-dye ion-pair complex

Methods	Nominal concentration (µg/ml)	RSD%			
		Robustness		Ruggedness	
		Variable alerted [a]			
		pH [b]	Volume of Dye[c]	Inter-analysts	Inter-instruments
BCG	4.0	1.02	1.35	1.84	1.32
	8.0	0.60	1.60	1.63	1.53
	12	1.20	1.94	1.20	1.07
BTB	4.0	0.80	1.46	1.76	1.38
	6.0	0.76	1.72	1.31	1.72
	8.0	1.10	1.06	1.50	1.10
BPB	4.0	1.24	1.50	1.32	1.36
	8.0	1.05	1.83	1.45	1.82
	16	0.86	1.14	1.60	1.29
BCP	4.0	0.80	1.46	1.76	1.38
	8.0	0.76	1.72	1.31	1.72
	12	1.10	1.06	1.50	1.10

[a] Mean of three determinations.
[b] pH (±0.2).
[c] The volumes of dye used were 2.0 ± 0.2 ml.

Table 4 Application of the standard addition method for the determination of SLG in dosage forms (tablets) using the proposed methods

Method	Taken drug (µg/ml)	Pure drug added (µg/ml)	Parkineast tablets (10 mg)		Tremogine tablets (10 mg)	
			Total found (µg/ml)	Recovery [a] (%) ± SD	Total found (µg/ml)	Recovery [a] (%) ± SD
	4.0	2.0	5.964	99.40 ± 0.50	5.934	98.90 ± 0.45
BCG		4.0	804.8	100.60 ± 0.70	7.964	99.55 ± 0.68
		6.0	9.93	99.30 ± 1.20	10.05	100.50 ± 1.40
	4.0	2.0	5.952	99.20 ± 0.70	5.97	99.50 ± 0.60
BTB		4.0	7.96	99.50 ± 1.20	8.072	100.90 ± 0.90
		6.0	9.88	98.80 ± 1.60	9.97	99.70 ± 1.50
	4.0	2.0	5.946	99.10 ± 0.34	5.994	99.90 ± 0.50
BPB		4.0	7.976	99.70 ± 0.70	7.96	99.50 ± 0.80
		6.0	10.04	100.40 ± 1.40	9.90	99.00 ± 1.60
BCP	4.0	2.0	5.976	99.60 ± 0.30	5.952	99.20 ± 0.40
		4.0	8.0	100.00 ± 0.50	8.048	100.60 ± 0.70
		6.0	9.90	99.0 ± 1.10	9.94	99.40 ± 1.30

[a] Average of six determinations

Table 5 Results of analysis of tablets by the proposed methods for the determination of SLG and statistical comparison with the reported method[19]

Samples	Recovery [a] (%) ± SD				
	Proposed Methods				Reported Method [23]
	BCG	BTB	BPB	BCP	
Parkineast tablets (10 mg)	99.77±0.72	99.17±0.35	99.73±0.65	99.53±0.50	99.50 ± 0.64
t-value [b]	0.63	1.01	0.56	0.08	
F-value [b]	1.27	3.34	1.03	1.64	
Tremogine tablets (10 mg)	99.65±0.80	100.03±0.76	99.46±0.45	99.73±0.76	99.70±0.70
t-value [b]	0.11	0.71	0.65	0.06	
F-value [b]	1.31	1.18	2.42	1.18	

[a] Average of six determinations.
[b] The theoretical values of *t* and *F* are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).

CONCLUSION

The proposed methods describe the application of extractive ion–pair complex formation reaction with dyes for the quantification of SLG in pure and dosage forms. Compared with the existing spectrophotometric methods, the proposed methods have the advantages of relatively simple, rapid, cost-effective, and more sensitive for determining SLG in pure and dosage forms. Moreover, the proposed methods are free from tedious experimental steps such as heating unlike the previously reported spectrophotometric methods cited earlier. The most attractive feature of these methods is its relative

freedom from interference by the usual diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. The statistical parameters and the recovery data reveal high precision and accuracy of the proposed methods besides being robust and rugged. Therefore, the validated method could be useful for routine quality control assay of SLG in pure and dosage forms.

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DECLARATION OF CONFLICT
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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