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A simple and reproducible estimation of some novel atypical antipsychotics by colorimetric method using bromocresol green as chromogen

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ABSTRACT

Quantification of three second generation atypical antipsychotics Iloperidone (ILO), Blonanserin (BLO) and Asenapine (ASE) have been demonstrated using a newly developed extractive spectrophotometric method. Structural characteristic of selected drugs allows them to form colored complex with acid dyes in presence of specific micro environment. Bromocresol green (BCG) was selected as dye. The colored complex has specific UV absorbance between 400 to 800 nm. Experimental conditions including buffer, pH of solution and solvent for extraction were optimized. Iloperidone and blonanserin were reacted with dye in presence of pH 1.2 hydrochloric acid buffer while asenapine was reacted in presence of pH 4.1 acetate buffer. The colored complex was extracted with chloroform and absorbance was measured that follows beer's low in range of 10-50 µg/mL for iloperidone (r^2 = 0.995) and blonanserin (r^2 = 0.998) and 5-30 µg/mL for asenapine (r^2 = 0.996). Proposed method was validated in terms of precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) as per the ICH guidelines. Results for inter-day precision (ILO < 2.20 %, BLO < 2.09 %, ASE < 2.43 %), intra-day precision (ILO < 1.74 %, BLO < 1.74 %, ASE < 1.24 %), % recovery (ILO = 98.05, BLO = 99.61, ASE = 98.60), LOD (ILO = 0.35, BLO = 2.41, ASE = 0.39 µg/mL), LOQ (ILO = 1.07, BLO = 7.31, ASE = 1.19 µg/mL) and stability of the drug-BCG complex (ILO = 120, BLO = 120, ASE = 60 min) were found satisfactory. The proposed analytical method is first, simple, reproducible and cost-effective having industrial application in qualitative and quantitative analysis.

Keywords: Antipsychotics, Iloperidone, Blonanserin, Asenapine maleate, Extractive colorimetric method

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INTRODUCTION

Schizophrenia is a chronic remitting and relapsing disease associated with shortened lifespan and significant impairment in social and vocational functioning due to symptoms like hallucinations, delusions and thought disturbances [1]. Over 60 antipsychotic medicines have been developed and classified in two groups first- and second-generation, "atypical" agents [1]. Iloperidone, a member of second generation antipsychotic belonging to piperidinyl-benzisoxazole derivatives, chemically defined as 4'-[3-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidino]propoxy]-3-methoxyacetophenone [2]. It was approved for the treatment of Schizophrenia in adults to Novartis by USFDA. Blonanserin, a second generation antipsychotic agent introduced by Dainippon Sumitomo for the treatment of schizophrenia in Japan was approved by PMDA [3]. Chemically, It belongs to butyrophenones and designated as 2-(4-ethylpiperazin-1-yl)-4-(4-fluorophenyl)-5, 6, 7, 8, 9, 10-hexahydrocycloocta [b] pyridine. Asenapine, another atypical anti-psychotic agent used in the treatment of schizophrenia, mania and bipolar disorder developed by Forest Pharmaceuticals was approved by USFDA [3]. It belongs to dibenzo-oxepino pyrroles and chemically defined as (3aRS, 12bRS)-5- Chloro-2-methyl-2, 3, 3a, 12b- tetrahydro-1*H*-dibenzo [2, 3:6, 7] oxepino [4, 5-c] pyrrole [4]. Various literatures on analytical methods for quantification of iloperidone, blonanserin and asenapine in its available market formulations using sophisticated instrumentation techniques were reported. Iloperidone estimation in human plasma using solid phase extraction followed by SIM-ES-MS method [5], RP-

UPLC method [6], HPLC methods [7-11], HPTLC method [19], UV spectrophotometric method [11, 13], and derivative spectrophotometric method [12] were reported. Blonanserin quantification was reported using LC-MS/MS method [14, 16] and UPLC-MS/MS method [15, 17] for its determination in human plasma [14], HPLC method [18, 20-21] spectrophotometric method [22]. Some UV spectrophotometric methods [23], HPLC methods [24, 29] and SPE-HPLC-MS method [30] were reported for estimation of asenapine. Recently simultaneous estimation of iloperidone with asenapine in human serum by LC-MS/MS method [31] and UPLC-MS method [32] were also reported.

In this paper, we present simple, reproducible, rapid and cost-effective ion-pair colorimetric method for quantitation of iloperidone, blonanserin and asenapine in bulk and its marketed formulation. Till date, there is no such colorimetric method reported for selected drug candidates in the literature. Method development by optimizing suitable microenvironment for complexation, validation of method as per the ICH guidelines [32], and industrial application of proposed method in quantification are the key points of our research.

MATERIALS AND METHODS

Apparatus

Double beam UV-visible spectrophotometer (Shimadzu, model 1700), quartz cells with 1 cm light path, digital balance (Shimadzu Libror AEG-220), digital pH meter (Systronic-335), ultrasonicator (Frontline FS-4), cyclomixer (Remi equipment), 1, 2, 5 and 10 mL pipettes, 10 and 25 mL volumetric flasks, 50 and 125 mL separating funnel and glass stoppered test-tubes were used during experiments.

Materials and reagents

Working standard of Iloperidone (ILO) was gifted by Themis Pharmaceuticals Ltd., Haridwar, India. Standards of Blonanserin (BLO) and Asenapine maleate (ASE) were gifted by Intas Pharmaceuticals Ltd., Ahmedabad, India. Hydrochloric acid buffers, Citrate buffers, acetate buffers and phosphate buffers of various pH ranges were prepared as mentioned in pharmacopoeia. Bromocresol green dye (BCG), anhydrous sodium sulfate, chloroform, dichloromethane, ethyl acetate, toluene, distilled water were used. All the reagents were of AR grade.

Preparation of standard stock solutions

Accurately weighed ILO (10 mg), ASE (10 mg) and BLO (10 mg) were transferred into three separate 10 mL volumetric flasks, dissolved and diluted up to the mark with methanol and stored in refrigerator at 2°-8°C wrapped with aluminium foil. Aliquots (2.5mL) were transferred into another set of 10 mL volumetric flasks and diluted up to the mark with distilled water to make working standard solutions (Concentration= 250 µg/mL for ILO, ASE and BLO respectively).

Preparation of acid dye solution

Accurately weighed Bromocresol green (100 mg) was transferred into 100 mL volumetric flasks. About 10 mL of methanol was added in the flasks; flasks was sonicated for 10 min to assist dissolution, and diluted with distilled water up to the mark. (Concentration= 1mg/mL).

Preparation of buffer solution

Hydrochloric acid buffers (pH 1.2 and 2.2), citrate buffers (pH 2.6, 2.8, 3.2, 3.6 and 3.8), acetate buffers (pH 4.1 and 5.5) and phosphate buffer pH 6.4 were prepared as per formulas listed in Pharmacopoeia.

Method development

Optimization of solvent for extraction of drug-dye complex and selection of wavelength

Working standard solution of ILO (1mL) was transferred into a series of different 25mL glass stoppered test-tubes. In each test-tube, 1mL of BCG dye solution was added along with 3 mL of 0.1N HCl buffer. The solutions were mixed well and 5 mL of selected organic solvents (chloroform, dichloromethane, ethyl acetate, and toluene) were added to respective test-tubes. The tubes were placed in cyclomixer for 2 min and were kept aside for layers to separate. From each test-tube, the organic layer was collected and filtered through anhydrous sodium sulfate to remove traces of water and was analyzed to determine its wavelength maximum and absorbance. The same procedure was adopted for ASE and BLO with same dye (BCG).

Optimization of pH for drug-dye complex formation

Standard stock solution of ILO (1mL) was transferred into a series of different 25mL glass stopper test-tubes. In each test-tube, 1mL of BCG dye solution was added along with 3 mL of different buffers (buffers of varying pH). The solutions were mixed well and 5mL of chloroform was added in each tube. The tubes were placed in cyclomixer for 2 min and were kept aside for layers to separate. The organic layers were collected and filtered through

anhydrous sodium sulfate (to remove traces of water). The same procedure was adopted for ASE and BLO. Absorbances of filtered solutions were measured at 419 nm for ILO, 415 nm for ASE and 417 nm for BLO.

Stochiometric determination of drug-dye complex by JOB'S curve method
Equimolar solution of drugs and dye (1.0×10^{-3}) were prepared in distilled water. Aliquots of drug solution (0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4 and 2.7mL) were transferred into a series of 25 mL glass stopper test tubes. To these tubes aliquots of dye solution (2.7, 2.4, 2.1, 1.8, 1.5, 1.2, 0.9, 0.6 and 0.3 mL respectively) were added in such a way that molar concentration of drug and dye remains constant while their mole fractions vary. To each test-tube 3 mL of buffer (HCL acid buffer pH 1.2 for ILO, Acetate buffer pH 4.1 for ASE and HCL acid buffer pH 1.2 for BLO) and 10 mL of chloroform was added. The tubes were placed in cyclomixer for 2 min and were kept aside for layers to separate. The organic layers were collected and filtered through anhydrous sodium sulfate to remove traces of water and the absorbance was measured at respective wavelength maximum. Absorbance which is proportional to complex formed was plotted against the mole fractions of these two components.

Reagent concentration
BCG dye was taken in concentration of 1 mg/ mL in order to allow excess of dye available for reaction irrespective to analyte concentration. Complexation is mole to mole reaction and ionized dye cannot be extracted by organic solvent hence, reaction and color intensity will remain unaffected.

Reaction time and numbers of extraction
During preliminary developmental trials, absorbance of drug–dye was measured with reaction time of 5 min and 30 min. No change in absorbance was noted. Reaction time of 10 min with intermittent shaking was sufficient to form the complex and complete the reaction. Single extraction with 10 mL of Chloroform is sufficient to extract out the drug-dye complex completely.

Stability of drug-dye complex
Stability of formed drug-dye complex was ascertained by continuously monitoring the absorbance values of the colored complexes after extraction using UV-spectrophotometer. Absorbance values were noted at an interval of five minutes till there was more than 2.0 % decrease in absorbance from its initial results. This time interval was termed as stability of the colored drug-dye complex.

Proposed method and assay of marketed formulation
[ILOSURE (Iloperidone), ASENAPT (Asenapine) and ELICIA (Blonanserin)]

For ILOSURE 2 (2 mg), ILOSURE 4 (4 mg)
Twenty tablets were accurately weighed and finely powdered. Powder equivalent to 10 mg ILO was transferred into 50 mL volumetric flask. To this, 40 mL preheated solution of methanol: distilled water (1:1) was added and the flask was sonicated for 20 min, the solution was filtered using Whatman filter no.41 and volume was made up to the mark with distilled water. Aliquot (1 mL) of this solution was transferred into a 25 mL glass stoppered test-tube followed by 3 mL of HCL acid buffer pH 1.2, 1 ml of BCG-dye and 7 mL with distilled water.

For ASENAPT 5 (5 mg), ASENAPT 10 (10 mg)
Twenty tablets were accurately weighed and finely powdered. Powder equivalent to 50 mg ASE was transferred into 50 mL volumetric flask. To this, 40 mL distilled water was added and the flask was sonicated for 10 min, the solution was filtered using Whatman filter no.41 and volume was made up to the mark with distilled water. Aliquot (1 mL) was transferred into a 10 mL volumetric flask and was diluted with distilled water up to the mark. Aliquot (1 mL) of this solution was transferred into a 25 mL glass stoppered test-tube followed by 3 mL of Acetate buffer pH 4.1, 1 ml of BCG-dye and 7 mL with distilled water.

For ELICIA 2 (2 mg), ELICIA 4 (4 mg)
Twenty tablets were accurately weighed and finely powdered. Powder equivalent to 10 mg BLO was transferred into 50 mL volumetric flask. To this, 40 mL distilled water was added and the flask was sonicated for 20 min, the solution was filtered using whatman filter no.41 and volume was made up to the mark with distilled water. Aliquot (2 mL) was transferred into a 25 mL glass stoppered test-tube followed by 3 mL of HCL acid buffer pH 1.2, 1 ml of BCG-dye and 7 mL with distilled water.

Extraction with chloroform was performed as described in validation studies (Linearity) for all the above test preparation and absorbance were measured in triplicates at respective wavelengths.

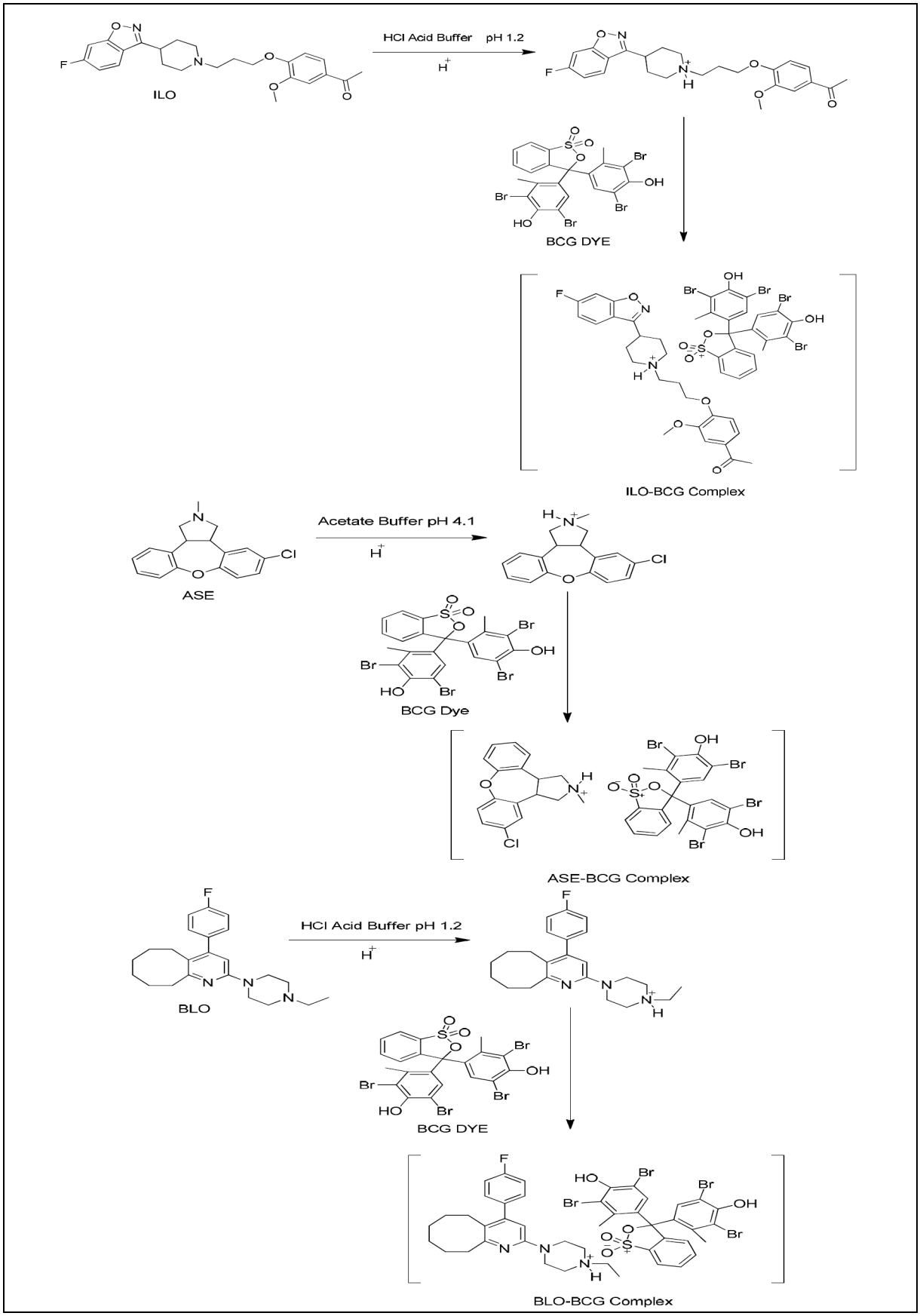

Figure 1 Reaction chemistry

RESULTS AND DISCUSSION

Complexation reaction chemistry

Quaternary amine in molecular structure of drugs undergoes ionization in presence of acidic pH. BCG dye forms anion at this pH. Yellow colored complex forms between cationic drug and anionic dye which is soluble in organic solvent and can easily be extracted. Complexation occurs at specific Stochiometric drug: dye ratio and excess of dye remains in ionized form and cannot be extracted. The reaction mechanism is depicted in figure-1.

Optimization of solvent for extraction of drug-dye complex

Organic solvent which can ensure the complete extraction of complex from the aqueous phase must be selected prior to method development. Amongst all tried organic solvents chloroform and dichloromethane was the first choice based on the absorbance as plotted in figure-2. Due to volatility of dichloromethane, chloroform was selected as the solvent for extraction.

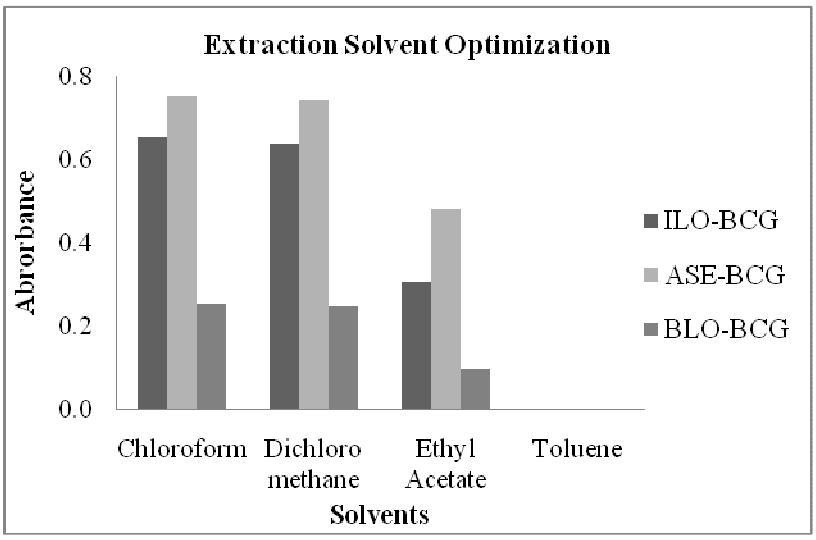

Figure-2 Absorbance of drug-dye complex in different organic solvents

UV spectrum and selection of wavelength

A spectrum of organic phase having yellow colored complex was recorded over a range of 400-800 nm to establish wave length maximum (λ_{max}). It was found to be 419 nm for ILO-BCG complex, 415 nm for ASE-BCG complex and 417 nm for BLO-BCG complex as depicted in figure-5. It can be observed that absorbance is specific to chromogen rather than analyte.

Optimization of pH for drug-dye complex formation

Micro-environment of the reaction mixture plays an important role in complexation reaction as pH governs the ionization. The results obtained during pH optimization studies showed that complexation reaction occurs at acidic pH. ILO and BLO forms complex at pH 1.2 whereas ASE forms complex at pH 4.1 (figure 3).

Stochiometric determination of drug-dye complex by Job's curve method

The results (figure 3) indicate that ion-pair forms when drug: dye ratio is 1:1 by electrostatic attraction between the positive protonated drug and the anionic dye. As observed from the Job's curve, absorption is maximum when drug/ [drug + dye] molar ratio is ~0.5 which indicates probability for complex formation in 1:1 (drug : dye) ratio for all the three drugs.

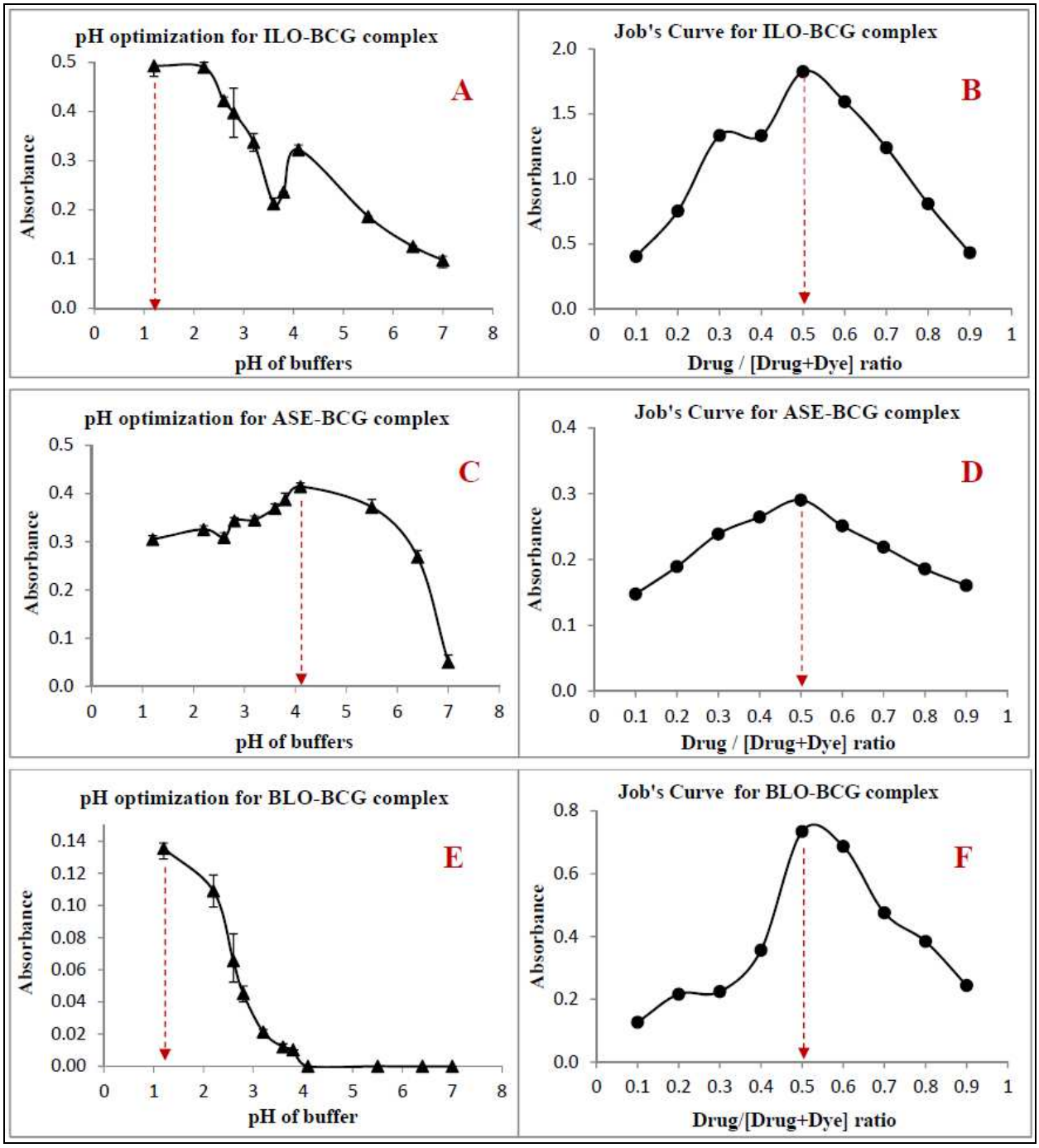

Figure-3: (A) ILO-BCG pH optimization (B) ILO-BCG Job's curve (C) ASE-BCG pH optimization (D) ASE-BCG Job's curve (E) BLO-BCG pH optimization (F) BLO-BCG Job's curve

Stability of drug-dye complex

Results for Stability of ILO-BCG, ASE-BCG and BLO-BCG complexes were presented graphically in figure-4. It was found to be stable till 150, 90 and 150 min respectively.

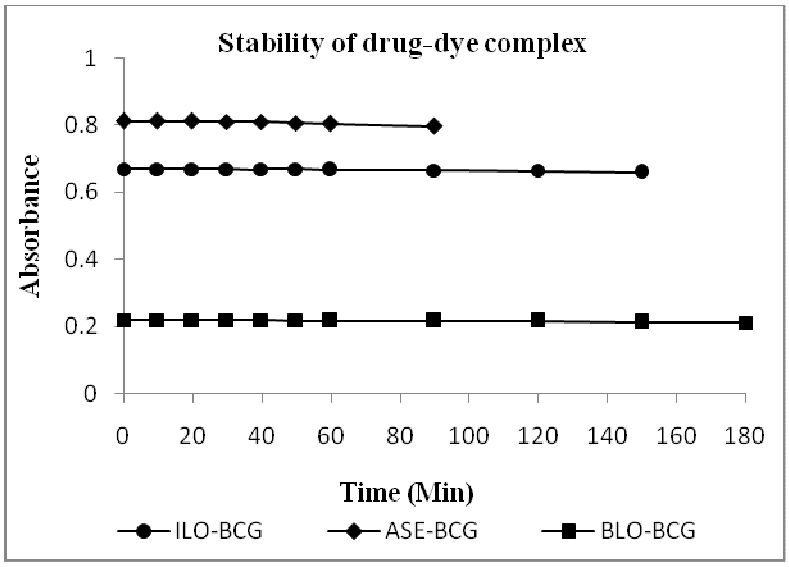

Figure-4 Stability of Drug-Dye complex after extraction

Method validation

The proposed methods were validated according to the current ICH guidelines [33]. Results of method validation studies were presented in table-1. Results were found satisfactory and within acceptance criteria.

Linearity (calibration curve)

For ILO: 2 mL working standard solution was transferred to 10 ml volumetric flask and diluted up to the mark (200µg/mL). Add resultant solution (0.5, 1.0, 1.5, 2.0 and 2.5mL) were transferred into a series of five 25 mL glass stopper test-tubes. In each test-tube 1 mL of BCG dye and 3 mL of HCL acid buffer pH 1.2 were added and the volume was made up to 7 mL with distilled water. Allow to stand for 10 min with intermittent shaking.

For ASE: 2.5 mL working standard solution was transferred to 10 ml volumetric flask and diluted up to the mark (250µg/mL). Add resultant solution (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL) were transferred into a series of five 25 mL glass stopper test-tubes. In each test-tube 1 mL of BCG dye and 3 mL of Acetate buffer pH 4.1 were added and the volume was made up to 7 mL with distilled water. Allow to stand for 10 min with intermittent shaking.

For BLO: Add 8 mL of Stock solution (250µg/mL) of standard BLO was transferred to 10 ml volumetric flask and diluted up to the mark (200µg/mL). Add resultant solution (0.5, 1.0, 1.5, 2.0 and 2.5mL) were transferred into a series of five 25 mL glass stopper test-tubes. In each test-tube 1 mL of BCG dye and 3 mL of HCL acid buffer pH 1.2 were added and the volume was made up to 7 mL with distilled water. Allow to stand for 10 min with intermittent shaking.

To the above solutions chloroform (10 mL) was added in each tube and was placed in cyclomixer to vortex for 2 min. The tubes were kept aside for layers to separate and the organic layer was collected, filtered through anhydrous sodium sulfate, UV visible spectrum of each solution was obtained and absorbance of colored ion pair complex was measured at respective wavelength maximum. This methodology was adopted in triplicates. Linearity plot was prepared and equation for linear regression was calculated (figure-1).

Intra-day and Inter-day precision

Intraday precision was determined at three different concentration levels of standard calibration curve for three times in the same day. Inter day precision was determined by analyzing three different concentration levels at three different days. Selected level of concentration were 10, 30, 50 µg/mL for ILO, 5, 15, 30 µg/mL for ASE and 10, 30, 50 µg/mL for BLO. Results were reported in terms of Coefficient of Variation (% CV).

Repeatability

To demonstrate repeatability of measurement of absorbance, a single concentration level of standard calibration curve was measured for seven times and Coefficient of Variation (CV) was reported. Selected levels of concentration were 30 µg/mL for ILO, 15 µg/mL for ASE and 30 µg/mL for BLO. Results were reported in terms of Coefficient of Variation (% CV).

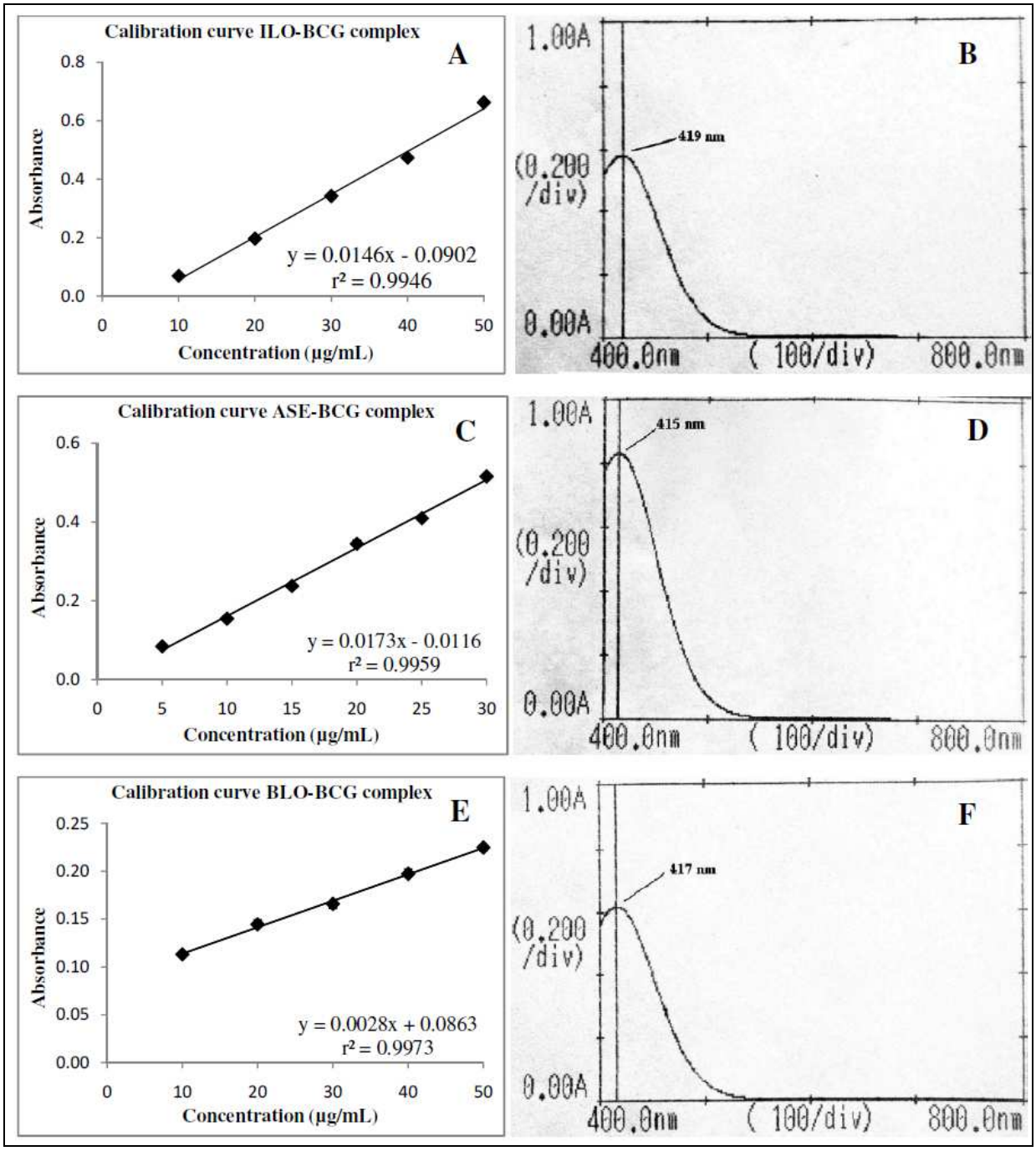

Figure-5 : (A) Calibration curve and (B) UV spectra of ILO-BCG complex, (C) Calibration curve and (D) UV spectra of ASE-BCG complex, (E) Calibration curve and (F) UV spectra of BLO-BCG complex

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined using following equation as per ICH guidelines:

LOD = 3.3 σ / S

LOQ = 10 σ / S

Where σ = S.D of y-intercept of calibration curves, S = Mean of slope of calibration curve

Accuracy

Accuracy of the analytical method is closeness of the result obtained by the method to the true value and was determined by recovery studies. Recovery was determined by spiking the known concentration of standard solution into the pre-analyzed market formulation sample at three levels within linearity range.

Table-1: Results of validation studies

Validation parameters	ILO		ASE		BLO	
Range (µg/mL)	10-50		5-30		10-50	
Regression equation (n=6)	y = 0.0146x - 0.0902		y = 0.0173x - 0.0116		y = 0.0028x + 0.0863	
Correlation coefficient (r^2)	0.9946		0.9959		0.9973	
Precision	Concentration µg/mL	% RSD	Concentration µg/mL	% RSD	Concentration µg/mL	% RSD
Intra-day (n=3)	10	1.74	5	1.04	10	1.74
	30	1.15	15	1.24	30	0.62
	50	0.92	30	0.81	50	0.09
Inter-day (n=3)	10	2.20	5	2.43	10	2.09
	30	1.56	15	1.26	30	1.30
	50	1.07	30	0.75	50	1.13
Repeatability (n=7)	30	0.96	15	1.17	30	1.50
Limit of detection (µg/mL)	0.35		0.39		2.41	
Limit of quantitation (µg/mL)	1.07		1.19		7.31	

Table-2: Results of assay obtained using proposed method

Marketed formulation	Marketed by	Labeled Claim	% Assay found[a]
ILOSURE 2 tablet	SUN Pharma	2 mg	99.35
ILOSURE 4 tablet		4 mg	100.13
ASENAPT 5 sublingual tablet	SUN Pharma	5 mg	99.04
ASENAPT 10 sublingual tablet		10 mg	99.34
ELICIA 2 tablet	Zydus Cadila Healthcare	2 mg	99.73
ELICIA 4 tablet		4 mg	101.61

[a] *average of three determinations*

Table -3: Results of recovery study using standard addition method

ILO				ASE				BLO			
Levels	Pure Added (µg)	Pure Found[a] (µg)	Amount Recovered (µg)	Levels	Pure Added (µg)	Pure Found[a] (µg)	Amount Recovered (µg)	Levels	Pure Added (µg)	Pure Found[a] (µg)	Amount Recovered (µg)
80	16	15.62	97.60	50	5	4.89	97.87	80	16	15.90	99.36
100	20	19.70	98.49	100	10	9.87	98.71	100	20	19.87	99.37
120	24	23.53	98.05	150	15	14.88	99.23	120	24	24.03	100.12
		98.05 ± 0.45				98.60 ± 0.68				99.61 ± 0.44	

[a] *average of three determinations*

CONCLUSION

The proposed colorimetric method is the first described method for the analysis of iloperidone, asenapine and blonanserin by ion-pair complexation reaction using bromocresol green as acid-dye in bulk and pharmaceutical preparations. Proposed methods are reliable considering the stability of ion-pair complex. Moreover proposed methods are cheaper since it can be performed using easily available laboratory reagents. Methods are specific and have no interference from common excipients that might be present in commercial formulations. It does not require any expensive instrumentation and critical reaction conditions. The method is simple, reproducible, accurate, precise and suitable for quality control and routine analysis of iloperidone, asenapine and blonanserin.

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