

## Original Article

**EXTRACTION-SPECTROPHOTOMETRIC DETERMINATION OF SOME ANTIHYPERTENSIVE DRUGS IN PHARMACEUTICAL AND BIOLOGICAL FLUIDS USING TWO SULPHONPHTHALEIN DYES**AKRAM M. EL-DIDAMONY<sup>1</sup>, SAMEH M. HAFEEZ<sup>2</sup>, AHMED A. SAAD<sup>1</sup><sup>1</sup>Chemistry Department, Faculty of Science, Zagazig University, Zagazig 44519, Egypt, <sup>2</sup>Ismailia Chemical Laboratory, Forensic Medicine Authority, Justice Ministry, Egypt  
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**ABSTRACT**

**Objective:** Two simple and sensitive extractive spectrophotometric methods have been described for the determination of some antihypertensive drugs namely, bisoprolol (BIS), carvedilol (CAR), propranolol (PRP) and telmisartan (TLM) either in pharmaceutical formulations or biological fluids.

**Methods:** The proposed methods involve the formation of yellow colored ion-pair complexes of the studied drugs with two sulphonphthalein dyes as bromophenol blue (BPB) and bromocresol purple (BCP) in acidic medium.

**Results:** The colored products are extracted into methylene chloride and measured spectrophotometrically at 402 nm for all the drugs. Beer's law limits, Sandell sensitivity values, limits of detection (LOD) and quantification (LOQ) values have also been reported for both the methods. The composition of the ion-pair complexes was found 1: 1 by Job's continuous variations method. The effects of concentration of dye, pH and interference of excipients have been studied and optimized. The accuracy and precision of the methods were evaluated on intra-day and inter-day basis; the relative standard deviation (RSD) was <1.72%. Various analytical parameters have been evaluated and the results have been validated by statistical data and indicated no significant difference in accuracy and precision.

**Conclusion:** The proposed methods were successfully applied to the determination of the studied drugs in pharmaceutical formulations and in biological fluids.

**Keywords:** Antihypertensive drugs, Sulphonphthalein dyes, Ion-pair, Pharmaceuticals.

**INTRODUCTION**

Bisoprolol (BIS, Scheme 1a) is a second generation selective  $\beta$ -blocker without intrinsic sympathetic activity. It is effective in reducing blood pressure [1, 2]. Several chromatographic methods have been reported for the analysis of BIS. Among the methods is high performance liquid chromatography (HPLC) [3-10], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [11] liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS) [12] and voltammetry [13] have been utilized for the determination of BIS. Few spectrophotometric methods have been reported in the literature for the determination of BIS in biological fluids and pharmaceutical preparations [14, 15].

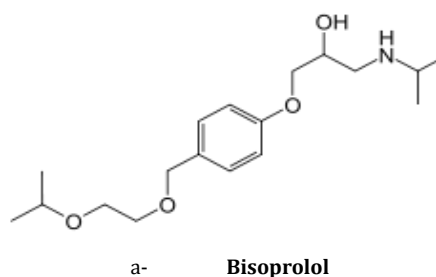
Carvedilol (CAR, Scheme 1b) is a non-selective and  $\beta$ -adrenergic antagonist with no intrinsic sympathomimetic activity and is widely used to treat essential hypertension and angina pectoris. [16-18]. Carvedilol is also indicated for the treatment of mild to severe chronic heart failure, Left ventricular dysfunction following myocardial infarction in clinically stable patients and hypertension. Beta blockers affect the heart and blood circulation [19-21]. Literature survey revealed that several methods such as spectrofluorimetry [22], spectrophotometry [23, 24], potentiometric [25], gas chromatography-mass spectrometry [26], liquid chromatography [27], HPLC and RP-HPLC [28-32] have been reported.

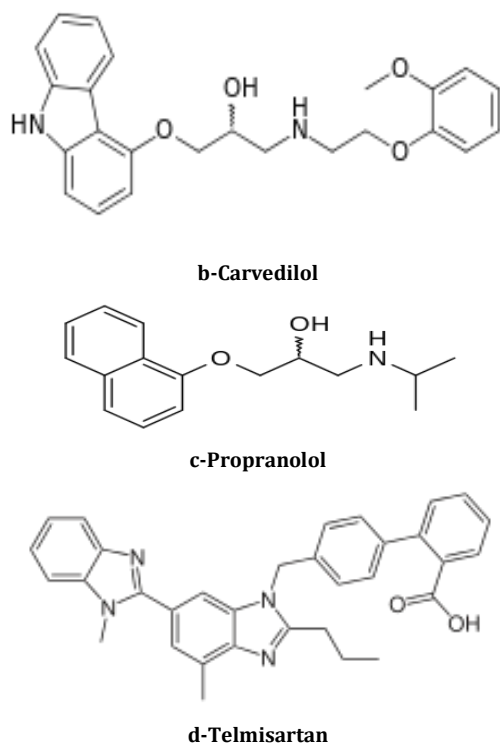
Propranolol (PRP, Scheme 1c), is a  $\beta$ -adrenergic receptor blocking agent that is prescribed for its antihypertensive, anti anxiety, anticonvulsant and antianginal effects. The analytical techniques used to determine propranolol in pharmaceuticals and in biological fluids such as HPLC [32, 33]. The other chromatographic methods, gas chromatography [34, 35], liquid chromatography [36, 37] and thin layer chromatography [38] are reported. Nonchromatographic methods such as UV derivative spectroscopy [39, 40], spectrofluorimetry [41-43], capillary electrophoresis [44-46],

polarography [47], voltammetry [48, 49] and spectrophotometry [50-53] are reported.

Telmisartan (TLM, Scheme 1d), an orally active angiotensin II antagonist acting on the AT1 receptor subtype [54]. Telmisartan is used for the treatment of hypertension (high blood pressure). It also is used for reducing the risk of heart attack, stroke, or death from cardiovascular causes in patients 55 years of age or older at high risk of developing major cardiovascular events who are unable to take ACE inhibitors [55]. Literature survey reveals that several methods like spectrophotometry [56, 57], HPLC and HPTLC [58-62] and voltammetry [63] were reported for the determination of telmisartan in combination with other drugs.

An attempt has been made to develop simple, economical, precise, accurate and reproducible spectrophotometric methods for estimation of BIS, CAR, PRP and TLM in bulk as well as pharmaceutical formulations. The proposed methods are based on the formation of ion-pair complex between the studied drugs with bromophenol blue (BPB) and bromocresol purple (BCP) in acidic medium. The constructed calibration curves were utilized in determining the concentration of these drugs in formulations and biological fluids.





Scheme 1: Chemical structure of the studied drugs

## MATERIALS AND METHODS

### Apparatus

All the absorbance spectral measurements were made using spectroskan 80 D double-beam UV/Visible spectrophotometer (Biotech Engineering Ltd. (UK), with wavelength range 190 nm ~ 1100 nm, spectral bandwidth 2.0 nm, with 10 mm matched quartz cells. The pH values of buffer solutions were measured using Jenway instrument pH-meter (combined electrode).

### Reagents and solutions

All of the chemicals used were of analytical or pharmaceutical grade and used without further purification. Double distilled de-ionized water was used to prepare all solutions.

Pharmaceutical grade of BIS, CAR, PRP and TLM certified to be 99.85% pure was obtained as gift was kindly supplied from Egyptian International Pharmaceutical Industries Company (EIPICO), Egypt. Stock solutions of pure BIS, CAR, PRP and TLM were prepared separately by dissolving accurately weighed 20 mg of each drug in a 100 ml calibrated flask. Working solutions of lower concentrations were freshly prepared by appropriate dilution with water. A  $1.0 \times 10^{-3}$  M of bromophenol blue and bromocresol purple (Aldrich Co., Ltd., Gillingham-Dorst, Germany), were prepared by dissolving 66.998 mg and 54.022 mg from each dye in 2 ml methanol then, add 20 ml distilled water and diluted to 100 ml in a calibrated flask with distilled water to the mark. Commercial dosage forms of BIS (10

mg/tablet Concor, product of Amoun Pharmaceutical Co., El-Obour city, Egypt), CAR (Carvid 7.5 mg Multi-apex, Badr-city, Cairo), PRP (Inderal 10 mg, EIPICO 10<sup>th</sup> of Ramadan, Egypt) and TLM (Micardis 40 mg, Boehringer Ingelheim Co., Germany). Series of buffer solutions of KCl-HCl (pH 1.0-2.2), NaOAc-HCl (1.99-4.92) and NaOAc-AcOH (3.4-5.6) pH was prepared by standard methods.

### General recommended procedures

#### Procedures for calibration curves

Into a series of separated funnels, accurately measured aliquots of BIS, CAR, PRP and TLM in the concentration range as shown in (table 1) were pitted out. A volume of 3.0 ml of  $1.0 \times 10^{-3}$  M BPB or BCP was added. Then, 2.0 ml of the buffer solution of pH = 2 was added in each case and the volume was completed to 10 ml with distilled water. The ion-pairs were extracted with 10 ml of methylene chloride by shaking for 2.0 min and then, the combined methylene chloride extracts were dried over anhydrous sodium sulphate. The absorbance of colored ion-pair complexes were measured within 20 min of extraction against the reagent blank prepared in the same manner except addition of drugs. In both the methods, a standard curve was prepared by plotting the absorbance values versus concentrations of drug. A linear equation for the standard curve was calculated by linear regression.

#### Procedure for tablets

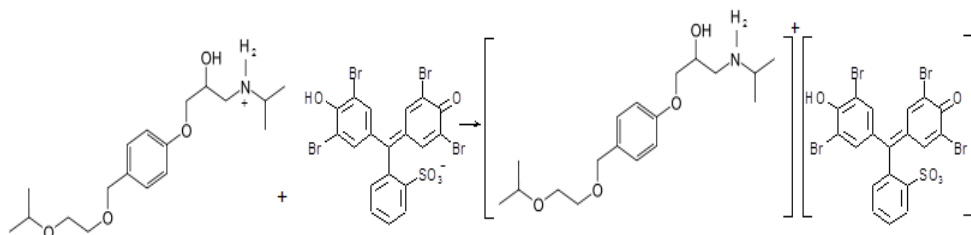
Ten tablets of each commercial pharmaceutical formulation for BIS, CAR, PRP and TLM were crushed, powdered, weighed out and the average weight of one tablet was determined. An accurate weight equivalent to 10 mg each drug and then active component was transferred into a 100 ml measuring flask. About 25 ml of distilled water was added and the mixture was shaken thoroughly for about 5 min. Then, it was diluted up to the mark with distilled water, mixed well and filtered using filter paper. An aliquot of this solution was diluted appropriately to obtain the working concentrations and analyzed as described under the standard procedure.

#### Procedures for human serum and urine

The proposed methods were applied to the determination of the studied drugs in spiked urine and serum provided from several healthy volunteers. Spiked urine was 5-fold diluted with distilled water. A 10 ml of serum sample was deproteinized by adding 5 ml of acetonitrile in a centrifuge for 5 min at 1000 rpm. The supernatant was used to investigate recovery. Add an aliquot of standard aqueous solution of each drug to 1.0 ml of diluted urine or serum. Proceed as described above. A blank value was determined by treating drug-free urine and drug-free serum in the same way. The absolute recovery was determined for each drug by comparing the representative absorbance of the treated urine or serum samples with the absorbance of the standard drug at the same concentration.

## RESULTS AND DISCUSSION

The proposed methods are based on the formation of methylene chloride soluble ion-pair complexes between the studied drugs and BPB or BCP in an acidic solution. The amino groups bind the proton more strongly than water molecules and that is the main driving force for the extraction. Different pHs show very different degrees of extraction under similar conditions and hence extraction also depends upon the anion [64, 65]. The suggested reaction pathway for the reaction product of BIS-BPB ion-pair complex formation for example, is given in Scheme 2.



Scheme 2: Suggested mechanism of BIS-BPB ion-pair complex formation

### Optimization of the reaction conditions

A number of preliminary experiments established optimum conditions necessary for rapid and quantitative formation of colored ion-paired complexes to achieve the maximum stability and sensitivity. Optimum condition was fixed by varying one parameter at a time while keeping other parameter constant and observing its effect on the absorbance.

### Spectral characteristics

The absorption spectra of the ion-pair complexes were measured in the range 300-550 nm against the blank solution as shown in fig. 1. The ion-pair complexes show maximum absorbance at 402 nm for all the drugs. The measurements were made at 402 nm for bulk, dosage forms and biological fluids. The colorless blanks have practically negligible absorbance.

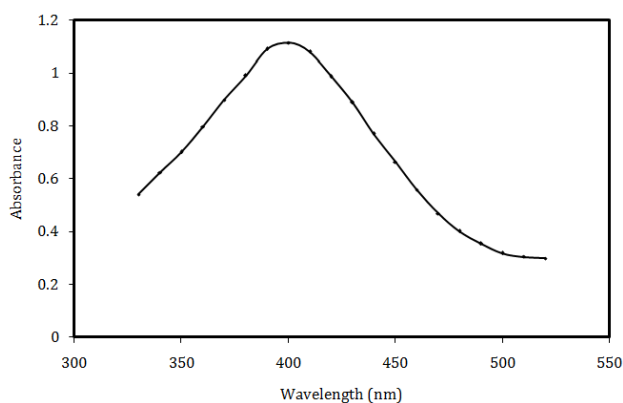


Fig. 1: Absorption spectra of BIS-BPB complex extracted in methylene chloride

### Effect of buffer type and pH

It was observed that the effective extraction of the complex depends on the type of buffer used and its pH. The effect of pH was studied by extracting the colored complexes in the presence of various buffers such as KCl-HCl (pH 1.0-2.2), NaOAc-HCl (pH 1.99-4.92) and NaOAc-AcOH (pH 3.6-5.6). It is evident that the maximum color intensity and maximum absorbance were found in KCl-HCl buffer. It was evident that the maximum absorbance of ion-pair complexes with BPB and BCP and minimum absorbance of the reagent blank was observed at pH of 2.0. At pH values greater than 2.0, a decrease in absorbance of the ion-pair complexes was observed. Hence the pH 2.0 was fixed in all subsequent measurements. Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5-5.0 ml). The higher absorbance value obtained at using 2.0 ml of buffer solutions.

### Effect of reagent concentration

The drug concentrations were kept constant, while the concentrations of BPB or BCP were varied from 0.5-4.0 ml of  $1.0 \times 10^{-3} M$ . The results showed that the absorbance of the extracted ion-pairs increased by increasing the BPB or BCP concentrations till 2.0 ml. After this volume, the absorbance remains constant by increasing the volume of the reagents. So any excess of reagents has no effect on the determination of the drugs.

### Choice of organic solvents

Different organic solvents as methylene chloride, carbon tetrachloride, chloroform and ether were tested as extractive solvents for the proposed methods. Methylene chloride was preferred to other solvents for its selective and obtained the highest absorbance with methylene chloride. It was also observed that only one extraction was adequate to achieve a quantitative recovery of the complexes and the shortest time to reach the equilibrium between both phases.

### Stability of the ion-pair complexes

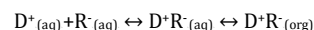
The stability of the ion-pair complexes formed between the studied drugs and BPB and BCP was evaluated. Although the ion-pairs were obtained instantaneously, constant absorbance readings were obtained after not less than 5.0 min of standing at room temperature ( $25 \pm 2^\circ C$ ). Ion-pairs were stable for at least 24 h without any change in color intensity or in  $\lambda_{max}$ .

### Effect of shaking time for extraction

Shaking time ranging from 0.5-4.0 min was tested to ascertain the extraction of the complex. Maximum and constant absorbance value were obtained when extracted after 1.5 min shaking. Therefore, shaking time of 2.0 min was maintained throughout the experiment.

### Composition of ion-pair complexes

In order to establish the molar ratio between BIS, CAR, PRP and TLM on one side and BPB or BCP reagent used on the other, Job's method of continuous variation was applied [66]. In this method,  $1.0 \times 10^{-3} M$  solutions of drugs and reagents was mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug (fig. 2). This procedure showed that a (1: 1) complex was formed through the electrostatic attraction between the positively charged drug,  $D^+$  ions and negatively charged reagent,  $R^-$  ions. The extraction equilibrium can be represented as follows:



Where  $D^+$  and  $R^-$  represent the protonated drug and the anion of the reagent, respectively and the subscripts "aq" and "org" refer to the aqueous and organic phases, respectively.

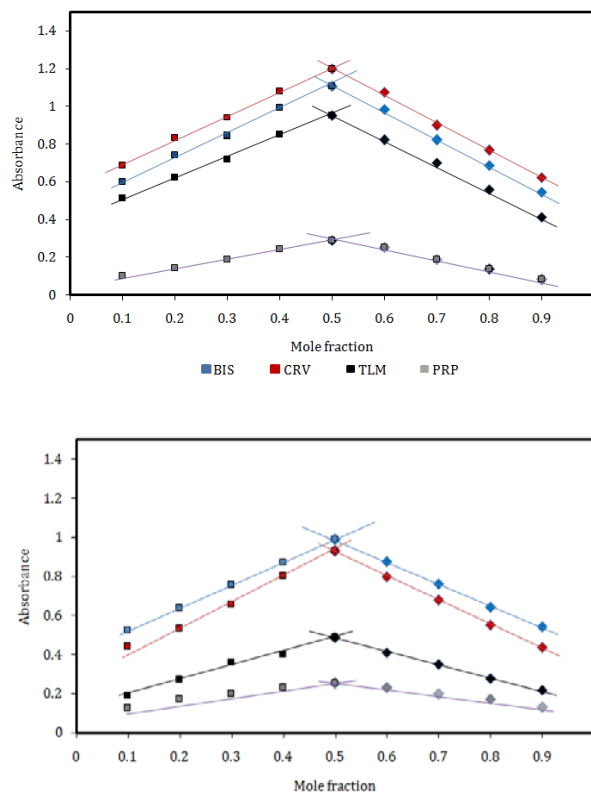


Fig. 2: Job's Continuous-variations plots of drug-dye systems

### Quantification

Under the optimum conditions described above, the absorbance-concentration plots were found to be linear over the concentration ranges stated in (table 1, fig. 3). The regression parameters given in the regression equation calculated from the calibration graphs along

with the standard deviations of the slope ( $S_b$ ) and the intercept ( $S_a$ ) are also given in table 1. 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 [67]. The apparent molar absorptivity, Sandell sensitivity, limits of detection and quantification of all the methods were also calculated and recorded in table 1. The BIS-BPB and CAR-BCP methods were found to be the most sensitive of all these methods with high  $\epsilon$  value.

#### Accuracy and precision

The precision of the proposed methods was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of the investigated drugs were analyzed in five replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and relative error (RE, %) of the found concentration compared to the theoretical one, respectively and indicate that the proposed method is highly accurate and reproducible (table 2, 3).

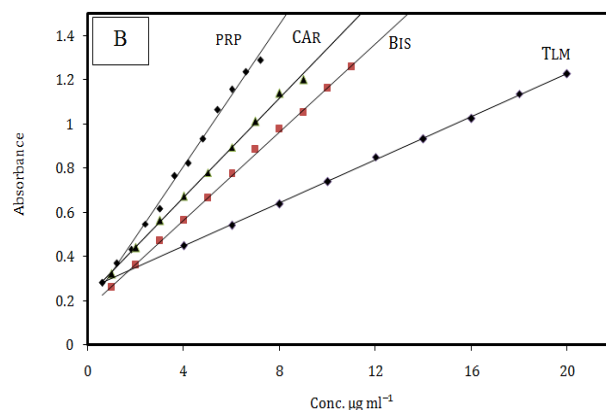
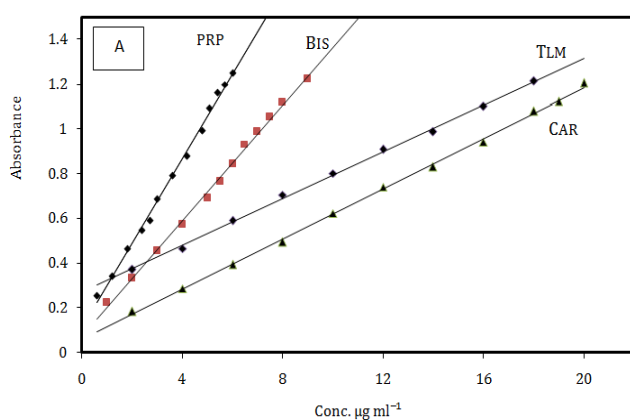


Fig. 3: Calibration curves of ion-pair complexes with: (a) BPB and (b) BCP

#### Analysis of dosage forms

To evaluate the validity and reproducibility of the proposed methods, known amounts of the studied drugs were added to the previously analyzed pharmaceutical preparations and the mixtures were analyzed by the proposed methods. The percent recoveries are given in table 4. Interference studies revealed that the common excipients and other additives such as lactose, starch, gelatin, talc and magnesium trisilicate, that are usually present in the tablet dosage forms did not interfere at their regularly added levels.

#### Analysis of biological fluids

The high sensitivity of the proposed methods, also allowed the *in vitro* determination of BIS, CAR, PRP and TLM in spiked human serum and urine samples. Thus the proposed methods are sufficient for routine estimation of the drugs in human serum and urine. The results obtained are satisfactorily accurate and precise (Tables 5, 6).

Table 1: Analytical parameters and optical characteristics for the studied drugs with BPB and BCP

| Parameters   | BPB   |                       |                        |                        | BCP                    |                        |                        |                        |
|--|---|-----------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
|  | BIS   | CAR                   | PRP                    | TLM                    | BIS                    | CAR                    | PRP                    | TLM                    |
| Beer's law limit, µg/ml                                  | 1.0-9.0   | 2.0-20.0              | 0.6-6.0                | 2.0-18.0               | 1.0-11.0               | 1.0-8.0                | 0.6-7.2                | 4.0-20.0               |
| Molar absorptivity, l mol <sup>-1</sup> cm <sup>-1</sup> | 6.96×10 <sup>4</sup>  | 2.73×10 <sup>4</sup>  | 3.23×10 <sup>4</sup>   | 3.56×10 <sup>4</sup>   | 5.71×10 <sup>4</sup>   | 7.52×10 <sup>4</sup>   | 2.82×10 <sup>4</sup>   | 3.32×10 <sup>4</sup>   |
| Sandell's sensitivity, ng/cm <sup>2</sup>                | 4.6   | 14.8                  | 4.3                    | 14.40                  | 5.6                    | 5.39                   | 4.9                    | 15.46                  |
| Correlation coefficient                                  | 0.9976  | 0.9999                | 0.9985                 | 0.9999                 | 0.9999                 | 0.9997                 | 0.9996                 | 0.9999                 |
| <b>Linear regression equation</b>                        | <b>A = a+bC, where A is the absorbance, a is the intercept, b is the slope and C is the concentration in µg/ml.</b> |                       |                        |                        |                        |                        |                        |                        |
| Intercept (a)  | 0.0450  | 0.0840                | 0.1103                 | 0.1941                 | 0.2124                 | 0.2031                 | 0.1609                 | 0.2456                 |
| Slope (b)  | 0.1340  | 0.0501                | 0.1935                 | 0.0574                 | 0.0852                 | 0.1223                 | 0.1642                 | 0.0491                 |
| $S_{y/x}$  | 12.29×10 <sup>-3</sup>  | 3.60×10 <sup>-3</sup> | 18.25×10 <sup>-3</sup> | 12.24×10 <sup>-3</sup> | 12.51×10 <sup>-3</sup> | 12.01×10 <sup>-3</sup> | 40.82×10 <sup>-3</sup> | 4.47×10 <sup>-3</sup>  |
| S. D. of slope ( $S_b$ )                                 | 2.4×10 <sup>-3</sup>  | 2.8×10 <sup>-4</sup>  | 4.8×10 <sup>-3</sup>   | 8.9×10 <sup>-4</sup>   | 1.94×10 <sup>-3</sup>  | 2.6×10 <sup>-3</sup>   | 9.69×10 <sup>-3</sup>  | 5.39×10 <sup>-4</sup>  |
| S. D. of intercept ( $S_a$ )                             | 33.4×10 <sup>-3</sup>   | 76.4×10 <sup>-4</sup> | 35.48×10 <sup>-3</sup> | 17.53×10 <sup>-3</sup> | 12.51×10 <sup>-3</sup> | 19.8×10 <sup>-3</sup>  | 80.66×10 <sup>-3</sup> | 15.91×10 <sup>-3</sup> |
| LOD, µg/ml   | 0.1791  | 0.9980                | 0.1583                 | 0.8711                 | 0.5868                 | 0.4088                 | 0.3045                 | 1.0183                 |
| LOQ, µg/ml   | 0.5964  | 3.3233                | 0.5271                 | 2.9006                 | 1.9542                 | 1.3614                 | 1.0140                 | 3.3910                 |

Table 2: Evaluation of intra-day accuracy and precision for the studied drugs with BPB and BCP

| Method | drug | Drug taken µg/ml | Drug found, µg/ml | Recovery <sup>a</sup> , % | RSD <sup>b</sup> , % | RE <sup>c</sup> , % |
|--------|------|------------------|-------------------|---------------------------|----------------------|---------------------|
| BPB    | BIS  | 4                | 3.99              | 99.999                    | 1.116                | -0.250              |
|        |      | 6                | 6.00              | 100.022                   | 0.716                | 0.022               |
|        |      | 8                | 7.99              | 99.999                    | 0.504                | -0.125              |
|        |      | 10               | 9.99              | 99.999                    | 1.111                | -0.001              |
|        | CAR  | 16               | 15.99             | 99.999                    | 1.123                | -0.001              |
|        |      | 20               | 19.97             | 99.899                    | 0.787                | -0.101              |
|        |      | 3                | 2.99              | 99.941                    | 0.457                | -0.059              |
|        |      | 4.2              | 4.19              | 99.977                    | 0.316                | -0.023              |
|        | PRP  | 5.4              | 5.39              | 99.982                    | 0.268                | -0.018              |
|        |      | 6                | 5.99              | 99.993                    | 1.722                | -0.007              |
|        |      | 12               | 11.99             | 99.996                    | 0.659                | -0.004              |
|        |      | 16               | 15.99             | 99.981                    | 0.933                | -0.019              |
| TLM    | 6    | 5.99             | 99.998            | 0.638                     | -0.166               |                     |

|     |     |     |       |        |       |        |
|-----|-----|-----|-------|--------|-------|--------|
| BCP | BIS | 8   | 7.99  | 99.999 | 0.759 | -0.125 |
|     |     | 11  | 10.99 | 99.999 | 0.344 | -0.091 |
|     |     | 3   | 2.99  | 99.999 | 1.528 | -0.001 |
|     | CAR | 5   | 4.99  | 99.998 | 1.220 | -0.002 |
|     |     | 7   | 6.99  | 99.997 | 0.587 | -0.003 |
|     |     | 3   | 2.99  | 99.999 | 1.012 | -0.001 |
|     | PRP | 4.8 | 4.79  | 99.999 | 0.703 | -0.001 |
|     |     | 6   | 5.99  | 99.998 | 0.734 | -0.002 |
|     |     | 8   | 7.99  | 99.999 | 1.682 | -0.001 |
|     | TLM | 14  | 13.99 | 99.999 | 0.643 | -0.001 |
|     |     | 18  | 17.99 | 99.994 | 0.909 | -0.006 |

<sup>a</sup>Mean value of five determinations; <sup>b</sup>Relative standard deviation (%); <sup>c</sup>Relative error (%).

**Table 3: Evaluation of inter-day accuracy and precision for the studied drugs with BPB and BCP**

| Method | drug | Drug taken µg/ml | Drug found, µg/ml | Recovery <sup>a</sup> , % | RSD <sup>b</sup> , % | RE <sup>c</sup> , % |        |
|--------|------|------------------|-------------------|---------------------------|----------------------|---------------------|--------|
| PBP    | BIS  | 4                | 3.99              | 99.999                    | 0.750                | -0.250              |        |
|        |      | 6                | 6.00              | 100.050                   | 0.399                | 0.050               |        |
|        |      | 8                | 7.99              | 99.999                    | 0.496                | -0.125              |        |
|        | CAR  | 10               | 9.99              | 99.999                    | 1.132                | -0.001              |        |
|        |      | 16               | 15.99             | 99.997                    | 0.674                | -0.003              |        |
|        |      | 20               | 19.99             | 99.999                    | 0.546                | -0.001              |        |
|        | PRP  | 3                | 3.00              | 100.059                   | 0.463                | 0.059               |        |
|        |      | 4.2              | 4.20              | 100.022                   | 0.398                | 0.022               |        |
|        |      | 5.4              | 5.40              | 100.017                   | 0.301                | 0.017               |        |
|        | TLM  | 6                | 6.00              | 100.033                   | 1.515                | 0.033               |        |
|        |      | 12               | 11.99             | 99.964                    | 0.713                | -0.083              |        |
|        |      | 16               | 15.99             | 99.999                    | 0.913                | -0.062              |        |
|        | BCP  | BIS              | 6                 | 5.99                      | 99.833               | 0.404               | -0.166 |
|        |      |                  | 8                 | 7.99                      | 99.999               | 0.550               | -0.125 |
|        |      |                  | 11                | 10.99                     | 99.999               | 0.344               | -0.091 |
|        |      | CAR              | 3                 | 2.99                      | 99.996               | 1.99                | -0.004 |
|        |      |                  | 5                 | 4.99                      | 99.998               | 0.793               | -0.002 |
|        |      |                  | 7                 | 6.99                      | 99.997               | 0.458               | -0.003 |
| PRP    |      | 3                | 2.99              | 99.999                    | 1.237                | -0.333              |        |
|        |      | 4.8              | 4.79              | 99.999                    | 0.853                | -0.001              |        |
|        |      | 6                | 5.99              | 99.999                    | 0.786                | -0.001              |        |
| TLM    |      | 8                | 7.99              | 99.999                    | 0.859                | -0.001              |        |
|        |      | 14               | 13.99             | 99.999                    | 0.643                | -0.001              |        |
|        |      | 18               | 17.99             | 99.998                    | 0.462                | -0.002              |        |

<sup>a</sup>Mean value of five determinations; <sup>b</sup>Relative standard deviation (%); <sup>c</sup>Relative error (%).

**Table 4: Recovery of the studied drugs in pharmaceutical formulation with BPB and BCP**

| Method | Drug | Drug formulation      | Drug taken µg/ml     | Drug found, µg/ml | Recovery <sup>a</sup> , % | RSD <sup>b</sup> , % | RE <sup>c</sup> , % |        |
|--------|------|-----------------------|----------------------|-------------------|---------------------------|----------------------|---------------------|--------|
| PBP    | BIS  | Concor 10 mg/tablet   | 4.0                  | 3.99              | 99.997                    | 1.116                | -0.003              |        |
|        |      |                       | 6.0                  | 6.00              | 100.022                   | 0.716                | 0.022               |        |
|        |      |                       | 8.0                  | 7.99              | 99.875                    | 0.504                | -0.125              |        |
|        | CAR  | Carvid 7.5 mg/tablet  | 10                   | 9.99              | 99.999                    | 1.111                | -0.001              |        |
|        |      |                       | 16                   | 15.99             | 99.997                    | 1.123                | -0.003              |        |
|        |      |                       | 20                   | 19.97             | 99.852                    | 0.787                | -0.148              |        |
|        | PRP  | Inderal 40 mg/tablet  | 3.0                  | 2.99              | 99.999                    | 0.858                | -0.001              |        |
|        |      |                       | 4.2                  | 4.19              | 99.999                    | 1.706                | -0.001              |        |
|        |      |                       | 5.4                  | 5.39              | 99.999                    | 1.675                | -0.001              |        |
|        | TLM  | Micardis 40 mg/tablet | 6                    | 5.99              | 99.998                    | 1.188                | -0.002              |        |
|        |      |                       | 12                   | 11.99             | 99.966                    | 1.125                | -0.034              |        |
|        |      |                       | 16                   | 15.99             | 99.998                    | 0.749                | -0.002              |        |
|        | BCP  | BIS                   | Concor 10 mg/tablet  | 6.0               | 5.99                      | 99.999               | 1.587               | -0.001 |
|        |      |                       |                      | 8.0               | 7.99                      | 99.998               | 1.261               | -0.002 |
|        |      |                       |                      | 11.0              | 10.99                     | 99.999               | 0.985               | -0.001 |
|        |      | CAR                   | Carvid 7.5 mg/tablet | 3                 | 2.99                      | 99.996               | 1.374               | -0.004 |
|        |      |                       |                      | 5                 | 4.99                      | 99.999               | 2.382               | -0.001 |
|        |      |                       |                      | 7                 | 6.99                      | 99.999               | 2.110               | -0.001 |
| PRP    |      | Inderal 40 mg/tablet  | 3.0                  | 2.99              | 99.998                    | 1.865                | -0.002              |        |
|        |      |                       | 4.8                  | 4.79              | 99.999                    | 1.075                | -0.001              |        |
|        |      |                       | 6.0                  | 5.99              | 99.874                    | 1.129                | -0.126              |        |
| TLM    |      | Micardis 40 mg/tablet | 8                    | 7.99              | 99.998                    | 1.828                | -0.002              |        |
|        |      |                       | 14                   | 13.99             | 99.999                    | 0.780                | -0.001              |        |
|        |      |                       | 18                   | 17.99             | 99.998                    | 0.751                | -0.002              |        |

<sup>a</sup>Mean value of five determinations; <sup>b</sup>Relative standard deviation (%); <sup>c</sup>Relative error (%).

Table 5: Recovery of the studied drugs in human serum with BPB and BCP

| Method | Drug | Drug taken µg/ml | Drug found, µg/ml | Recovery <sup>a</sup> , % | RSD <sup>b</sup> , % | RE <sup>c</sup> , % |
|--------|------|------------------|-------------------|---------------------------|----------------------|---------------------|
| PBP    | BIS  | 4                | 3.99              | 99.750                    | 1.418                | -0.250              |
|        |      | 6                | 6.00              | 100.054                   | 1.130                | 0.054               |
|        |      | 8                | 7.99              | 99.975                    | 1.565                | -0.025              |
|        | CAR  | 10               | 9.99              | 99.999                    | 1.357                | -0.001              |
|        |      | 16               | 15.98             | 99.937                    | 1.130                | -0.063              |
|        |      | 20               | 19.99             | 99.995                    | 0.444                | -0.005              |
|        |      | 3.0              | 3.00              | 100.077                   | 1.446                | 0.077               |
|        |      | 4.2              | 4.19              | 99.999                    | 1.034                | -0.001              |
|        | PRP  | 5.4              | 5.39              | 99.976                    | 1.064                | -0.024              |
|        |      | 6                | 5.99              | 99.999                    | 3.940                | -0.001              |
|        |      | 12               | 12.03             | 100.295                   | 0.970                | 0.295               |
|        | TLM  | 16               | 15.99             | 99.999                    | 0.839                | -0.001              |
|        |      | 6                | 5.99              | 99.952                    | 0.928                | -0.048              |
|        |      | 8                | 7.99              | 99.975                    | 0.491                | -0.025              |
|        | BCP  | BIS              | 11                | 10.99                     | 99.961               | 0.777               |
| 3      |      |                  | 2.99              | 99.968                    | 2.165                | -0.032              |
| 5      |      |                  | 4.99              | 99.965                    | 1.836                | -0.035              |
| CAR    |      | 7                | 6.99              | 99.998                    | 1.149                | -0.002              |
|        |      | 3.0              | 2.99              | 99.999                    | 2.821                | -0.001              |
|        |      | 4.8              | 4.79              | 99.996                    | 2.399                | -0.004              |
| PRP    |      | 6.0              | 5.99              | 99.996                    | 1.363                | -0.004              |
|        |      | 8                | 7.99              | 99.985                    | 1.694                | -0.015              |
|        |      | 14               | 13.99             | 99.996                    | 1.184                | -0.004              |
| TLM    |      | 18               | 17.99             | 99.992                    | 0.822                | -0.008              |

<sup>a</sup>Mean value of five determinations; <sup>b</sup>Relative standard deviation (%); <sup>c</sup>Relative error (%).

Table 6: Recovery of the studied drugs in urine with BPB and BCP

| Method | Drug | Drug taken µg/ml | Drug found, µg/ml | Recovery <sup>a</sup> , % | RSD <sup>b</sup> , % | RE <sup>c</sup> , % |
|--------|------|------------------|-------------------|---------------------------|----------------------|---------------------|
| PBP    | BIS  | 4.0              | 3.99              | 99.965                    | 2.760                | -0.035              |
|        |      | 6.0              | 5.99              | 99.921                    | 1.704                | -0.079              |
|        |      | 8.0              | 8.00              | 100.037                   | 0.958                | 0.037               |
|        | CAR  | 10               | 10.00             | 100.080                   | 1.236                | 0.080               |
|        |      | 16               | 16.00             | 100.050                   | 2.175                | 0.050               |
|        |      | 20               | 20.00             | 100.015                   | 0.506                | 0.015               |
|        |      | 3.0              | 3.00              | 100.065                   | 1.562                | 0.065               |
|        |      | 4.2              | 4.20              | 100.079                   | 1.097                | 0.079               |
|        | PRP  | 5.4              | 5.40              | 100.039                   | 1.638                | 0.039               |
|        |      | 6                | 5.99              | 99.966                    | 1.289                | -0.034              |
|        |      | 12               | 12.02             | 100.200                   | 1.075                | 0.200               |
|        | TLM  | 16               | 15.99             | 99.955                    | 0.489                | -0.045              |
|        |      | 6.0              | 5.99              | 99.999                    | 2.710                | -0.001              |
|        |      | 8.0              | 7.99              | 99.999                    | 1.522                | -0.001              |
|        | BCP  | BIS              | 11.0              | 11.00                     | 100.006              | 2.208               |
| 3      |      |                  | 2.99              | 99.998                    | 2.465                | -0.002              |
| 5      |      |                  | 4.99              | 99.999                    | 2.238                | -0.001              |
| CAR    |      | 7                | 6.99              | 99.991                    | 2.108                | -0.009              |
|        |      | 3.0              | 2.99              | 99.996                    | 1.0338               | -0.004              |
|        |      | 4.8              | 4.79              | 99.998                    | 2.798                | -0.002              |
| PRP    |      | 6.0              | 5.99              | 99.998                    | 2.356                | -0.002              |
|        |      | 8                | 7.99              | 99.999                    | 1.126                | -0.001              |
|        |      | 14               | 13.99             | 99.999                    | 1.526                | -0.001              |
| TLM    |      | 18               | 17.98             | 99.944                    | 1.241                | -0.056              |

<sup>a</sup>Mean value of five determinations; <sup>b</sup>Relative standard deviation (%); <sup>c</sup>Relative error (%).

## CONCLUSION

Spectrophotometric techniques continue to be the most preferred method for routine analytical work because of its simplicity and reasonable sensitivity with significant economical advantages. The proposed methods make use of the simple reagent which an ordinary analytical laboratory can afford and the procedures do not involve any critical reaction conditions or tedious sample preparation. The methods are highly reliable owing to the stability of the ion-pair complex and acid/base forms of the dye, which are ultimately measured. The methods were successfully applied to the pharmaceutical formulations, spiked human serum and urine. All the developed methods may be recommended for routine and quality

control analysis of the investigated drugs in pharmaceutical preparations.

## CONFLICT OF INTERESTS

Declared None

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