Extractive Spectrophotometric Methods for Determination of Chlorpheniramine Maleate in Pure Form, Pharmaceutical Preparations and Biological Fluids

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Abstract. In this study a simple, rapid and sensitive spectrophotometric method was developed for the determination of an antihistamine drug chlorpheniramine maleate (CPM) in pure form, pharmaceutical preparations, spiked humane urine and spiked blood serum. This method was based on the formation of ion-pairs between the basic nitrogen of the CPM drug and four chromogenic reagents namely bromocresol purple (BCP), alizarine Red S (ARS), eriochrome cyanine R (ECR), and cresol red (CR). The extracted colored ion-pairs were measured spectrophotometrically at 390, 425, 503 and 408 nm for BCP, ARS, ECR and CR reagents, respectively. The different parameters that affect the color development between CPM drug and dyestuff reagents were extensively studied to determine the optimal conditions for the assay procedure. The reaction was studied as a function of the volume of reagents, nature of solvent, temperature, reaction time and stoichiometric ratio between the CPM drug and the reagents. Beer’s law was valid over the concentration ranges of 1-30, 1-10, 2-120 and 4-120 μg mL\textsuperscript{-1} of CPM drug using BCP, ARS, ECR and CR reagents, respectively. The Sandell sensitivity, molar absorptivity, limit of detection and limit of quantification were determined. Applications of the proposed procedure to the analysis of the pharmaceutical preparations, spiked humane urine and spiked blood serum gave reproducible and accurate results without any interference from excipients. The results obtained by the proposed method were in good agreement with those obtained by reported method. The method can be suggested for the routine analysis of the cited drug.

1. Introduction

The structure of chlorpheniramine maleate (CPM) was given as shown in Fig. 1. Chlorpheniramine maleate effective against motion sickness and nausea, with its primary mechanism of action being its activity to decrease the levels of acetylcholine in the brain [1]. CPM drug inhibits the impacts of histamine on capillary penetrability and bronchial smooth muscles. It is an anti-allergic drug, generally in cough-cold preparations. CPM drug has been determined either alone or in combination using a number of techniques including chromatography [1, 3-8] and spectrophotometry [2, 9-14]. These methods are time-consuming, expensive and boring. Also, they need separation steps and high laboratory skills. It is necessary to find a more effective, economical and specific method as an alternative. So dyestuff reagents were used for determination of CPM drug [15-24] through formation of ion pairs between CPM drug and some selected dye stuff reagents. The aim of this work was to find a fast, simple and accurate extractive spectrophotometric method for the determination of CPM drug in pure form and pharmaceutical preparations available in Egyptian markets, spiked human urine and spiked blood serum samples. The proposed method was depend on the reaction of CPM drug and anionic reagents namely BCP, ECR, ARS and CR to produce ion pairs with intense color which can be absorbed in visible region which were extracted and then measured at the optimum wavelength. The different parameters that affect the color development between CPM drug and reagents were studied to determine the optimal conditions for the assay procedure.
2. Experimental

2.1. Materials and reagents

All reagents and chemicals used were of analytical grade, and the solvents used were of spectroscopic grade. The CPM drug was provided from Misr Company for Pharmaceutical Industry. Dyestuffs included bromocresol purple (BCP, 99%), alizarin Red S (ARS, 99%), eriochrome cyanine R (ECR, 99%) and cresol red (CR, 99%). They were purchased from Win lab, UK. Allergy tablets (4 mg chlorpheniramine maleate per tablet) was purchased from Arab drug company (Adco). Absolute ethanol was supplied from Adwic. Chloroform, methanol, ethanol, acetone, ethylene chloride and methylene chloride were supplied from El-Nasr Company, Egypt. Acetonitrile was supplied from Fisher chemicals.

2.2. Solutions

0.02% (w/v) of BCP and CR solutions were prepared by dissolving the appropriate weight in 30 : 70 (v/v) ethanol : water mixture. 0.1% (w/v) ARS and ECR solutions were prepared by dissolving the appropriate weight in 100 mL distilled water. 0.1% (w/v) CPM solution was prepared by dissolving the appropriate weight in 100 mL distilled water. All solutions were stored in dark glass bottles to be protected from light. Drug-free human urine and blood serum were obtained from a healthy female aged about 23 years, from Hospital of Menuofia University.

2.3. Apparatus

The spectrophotometric measurements were carried out using UVmini-1240 shimadzu and 1cm quartz cell, with wavelength range of 190-1100 nm.

3. Procedure

3.1. Determination of the optimum wavelength

Into 5 mL volumetric flask, definite amount of dyestuff reagents and CPM drug were added to each other. Then these solutions were adjusted to the mark with distilled water. These solutions were left for 5 min at room temperature then were transferred into 100 mL separating funnel. The ion-pairs were extracted with chloroform twice with 5 mL in each time after shaking for 1 min for BCP and ARS reagents. But for ECR and CR reagents, the extraction was done in only 5 mL chloroform. Then the ion-pairs were collected in measuring flask. Full scan was done for each ion pair and maximum wavelength was determined.

3.2. Effect of time and temperature

To select the optimum time and temperature for ion-pair formation, 0.2-0.5 mL of 2.6 x 10^{-3} mol L^{-1} of CPM drug was added to 1 mL of BCP, CR, ARS or ECR reagents in 5 mL volumetric flask. The volumes were completed to the mark with distilled water. Then the mixture was extracted with chloroform for BCP, ECR and CR reagents and dichloromethane for ARS reagent. The absorbance was measured at $\lambda_{\text{max}}$ at different time intervals in the range from 0 to 60 minutes and at different temperatures in the range from 5 to 50 °C.
3.3. Procedure for tablets

25 Tablets of allergy were ground to fine powder and the weight equivalent to one tablet was dissolved in distilled water. The resulting solution was shaken well, filtered through filter paper and washed with distilled water. The filtrate and washings were collected in 100 mL measuring flask. Aliquots of filtrate were studied using the proposed procedure mentioned above. The concentration of CPM drug in the analyzed samples was calculated using either the calibration graph or the corresponding regression equation.

3.4. Procedure for spiked human urine and blood serum

The general procedure described above can be used to determine CPM drug concentration in spiked human urine and blood serum samples using standard graph or from the regression equation.

4. Results and Discussion

4.1. Absorption spectra

BCP, ECR, ARS or CR dye reagents reacted with CPM drug and gave intense colored ion-pairs extracted into chloroform. The maximum absorbance was obtained at 395, 425, 503 and 408 nm for BCP, ARS, ECR, and CR dye reagents, respectively, as shown in Fig. 2.

![Figure 2. Absorption spectra of ion-pairs.](image)

4.2. Effect of time

The optimum time for the completion of the ion-pairs formation between the dye reagents and CPM drug was found to be 5 minutes for BCP and ECR reagents and 10 minutes for ARS and CR reagents Fig. 3a. The absorbance values remain almost unchanged with the increase of time.

4.3. Effect of temperature

The effect of temperature on the ion pair formation between reagents and CPM drug has been studied and optimized. The absorbance of the extracted ion–pairs was measured in the temperature range from 5 to 50 °C. It was found that the absorbance increased with the increase of temperature and attains maximum values at room temperature of 25±2°C for BCP, ARS, ECR and CR reagents, as shown in Fig. 3b. It was found that the ion pairs were stable at high temperatures as indicated from the stability of the absorbance values.
4.4. Selection of the suitable extracting solvent

The effect of the extracting solvent used both on extraction efficiency and color intensity was studied. Absorbance values obtained using different solvents like chloroform, dichloromethane and 1, 2-dichloroethane was measured. Chloroform was selected due to the more stability of the extracted colored ion pairs for BCP, ECR and CR reagents and methylene chloride for ARS reagent, as shown in Fig. 3c. Table 1 showed the obtained absorbance and the respective molar absorptivity values of the ion pairs using different extractive solvents.

Figure 3. Effect of different parameters on the formation of the ion pairs of CPM drug with BCP, ARS, ECR and CR reagents (a) time, (b) temperature and (c) organic solvent.
**Table 1.** The absorbance values and molar absorptivity for the determination of CPM drug using BCP, ARS, ECR and CR reagents in different organic solvents.

| Solvents          | Absorbance (A) | Molar absorptivity (ε) |
|-------------------|----------------|------------------------|
|                   | BCP | ARS | ECR | CR  | BCP | ARS | ECR | CR  |
| Chloroform        | 1.384 | 0.716 | 0.821 | 0.468 | 1.4x10^4 | 2.8x10^3 | 3.2x10^3 | 4.6x10^4 |
| Dichloromethane   | 1.203 | 0.808 | 0.290 | 0.436 | 1.2x10^4 | 3.2x10^3 | 1.1x10^3 | 4.3x10^4 |
| 1,2-Dichloroethane| 1.023 | 0.641 | 0.340 | 0.361 | 1.0x10^4 | 2.5x10^3 | 1.3x10^3 | 3.5x10^3 |

### 4.5. Effect of organic solvent extraction power:

The effect of varying the number of portions of the solvents on the quantitative extraction of the ion pairs was shown in Fig. 4a. It was concluded that reproducible absorbance readings were obtained after double extraction of ion pairs formed using BCP, ASR, ECR and CR reagents.

### 4.6. Effect of reagents volume

The effect of reagent volume was studied by adding different amount of dye to the same concentration of CPM drug and the absorbance of the formed ion pair solutions were measured. Maximum color intensity of ion pairs was achieved with 0.5 mL of dye solutions for ECR reagents and with 1 mL of dye solutions for ARS, CR and BCP reagents as shown in Fig. 4b. Excess volume of reagents had no effect on ion pair formation.

**Figure 4.** Effect of (a) solvent extraction power and (b) volume of dyestuff reagents on the absorbance of ion pairs.
4.7. Stoichiometric relationship

The stoichiometry of the ion-associates formed between the CPM drug under study was determined by applying the molar ratio and Job’s continuous variation methods [25] at the wavelengths of maximum absorbance. The results obtained were shown in Figs. 5a, b and indicated that the composition of the ion pairs (due to the electrostatic attraction between the positively charged drug and the used anionic reagents) was (1:1) (drug: reagent). This stoichiometric ratio supported that the interaction between the CPM drug and the reagents used took place at only one site of the CPM drug which was the more active as shown in Scheme 1.

![Graph a](image1.png)

![Graph b](image2.png)

**Figure 5.** Stoichiometric ratio using (a) molar ratio and (b) continuous variation methods graphs for the ion-pair reaction of CPM drug with dye reagents.
4.8. Validity of Beer’s law

Table 2 showed the results of studying the quantitiveness of the reaction between CPM drug and BCP, ARS, ECR and CR reagents under the selected optimum conditions in pure form. It was found that Beer’s law was valid over the concentration ranges of 1-30, 1-10, 2-120 and 4-120 μg mL⁻¹ of CPM drug with BCP, ARS, ECR, and CR reagents, respectively (Table 2). Also the slope, intercept, correlation coefficient, Sandell sensitivities, molar absorptivity (ε), standard deviation (SD), relative standard deviation (RSD), limits of detection (LOD) and quantification (LOQ) were presented in Table 2. The low values of Sandell sensitivity indicated the high sensitivity of the proposed method in the determination of the CPM drug under investigation. Five replicate measurements were performed at different concentrations of CPM drug using BCP, ARS, ECR, and CR reagents. The low values obtained indicate high precision and accuracy of the proposed spectrophotometric method. The low values of the limits of detection (LOD) and quantification (LOQ) indicated the possibility of applying BCP, ARS, ECR and CR reagents in routine analysis for quantitative determination of the CPM drug under investigation.

**Scheme 1.** The mechanism of interaction of CPM drug with dye reagents.
Table 2. Analytical parameters for the determination of CPM drug using BCP, ARS, ECR and CR reagents.

| Parameter                        | BCP | ARS | ECR | CR |
|----------------------------------|-----|-----|-----|----|
| λ<sub>max</sub> (nm)             | 395 | 425 | 503 | 408|
| t (min)                          | 5   | 10  | 5   | 10 |
| T(°C)                            | 25±2| 25±2| 25±2| 25±2|
| ε, L mol<sup>-1</sup> cm<sup>-1</sup> | 1.4x10<sup>4</sup> | 3.2x10<sup>3</sup> | 3.2x10<sup>3</sup> | 4.6x10<sup>3</sup> |
| Sandell Sensitivity, μg cm<sup>-2</sup> | 0.050 | 0.156 | 0.377 | 0.033 |
| Beer’s law limit, μg mL<sup>-1</sup> | 1-30 | 1-10 | 2-120 | 4-120 |
| Percentage recovery, %           | 98.40–101.3 | 98.50–100.1 | 98.00–100.8 | 99.10–102.5 |
| Relative Standard Deviation,(RSD)% | 0.42-1.13 | 0.33-0.75 | 0.60-1.6 | 0.28-1.70 |
| Regression equation *, slope (b) | 0.047 | 0.064 | 0.006 | 0.008 |
| Intercept (a)                    | 0.135 | -0.055 | 0.001 | 0.084 |
| Correlation coefficient (r²)     | 0.9979 | 0.9976 | 0.9981 | 0.9991 |
| LOD, μg mL<sup>-1</sup>          | 0.71 | 0.66 | 0.69 | 1.50 |
| LOQ, μg mL<sup>-1</sup>          | 2.40 | 2.20 | 2.30 | 5.00 |

*A = a + bC; as C is the concentration in μg mL<sup>-1</sup>, (n=5).

5. Comparison with Previous Reported Method

In order to evaluate the analytical applicability of the proposed spectrophotometric method, the results obtained by the proposed spectrophotometric method were compared with those of the HPLC reported method [4] and the results obtained were listed in Table 3. It was found that the proposed method was applicable for a wide concentration range, accurate and precise method.

Table 3. Comparison between the proposed and reported methods.

| Reagent | Drug taken μg mL<sup>-1</sup> | Proposed method | Reported method | F-test | t-Test |
|---------|-------------------------------|-----------------|-----------------|--------|--------|
|         | Found μg mL<sup>-1</sup> | Recovery (%) | SD | RSD (%) | Found μg mL<sup>-1</sup> | Recovery (%) | SD | RSD (%) |        |
| CR      | 120.0 | 118.9 | 99.10 | 0.07 | 0.66 | 119.0 | 99.20 | 0.03 | 0.28 | 5.44 | 1.90 |
| ARS     | 1.000 | 1.007 | 100.7 | 0.01 | 2.45 | 1.015 | 101.5 | 0.05 | 2.55 | 0.04 | 1.79 |
| ECR     | 60.00 | 59.82 | 99.70 | 0.04 | 1.10 | 59.78 | 99.63 | 0.09 | 1.15 | 0.19 | 2.23 |
| BCP     | 10.00 | 9.990 | 99.90 | 0.01 | 0.21 | 9.980 | 99.80 | 0.03 | 1.32 | 0.11 | 2.24 |

Tabulated F value at 95% confidence level is 6.39 (n=5)
Tabulated t value at 95% confidence level is 2.776 (n=5)

6. Method of Validation

6.1. Accuracy and precision

Accuracy can be defined as the closeness between the true or accepted value and the obtained value. The results in Table 2 showed high accuracy of the proposed method. While precision can be defined as the closeness of the results to each other. Precision expressed as standard or relative standard deviations of the replicate analysis [26].

6.2. Limit of detection and limit of quantification

LOD is the lowest quantity of the investigated compound in a sample that can be measured, but not necessarily quantified with an acceptable uncertainly. LOQ is the lowest amount of compound that can be detected in the sample at an acceptable level of accuracy and precision. The detection limit (LOD) for the proposed methods was determined using the following equation [27]:

\[ LOD = 3s/k \]
where the standard deviation of the intercept and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance were calculated and listed in Table 2. The limits of quantitation, LOQ, defined as [26]:

$$\text{LOQ} = 10s/ k .$$

According to this equation, the limit of quantitation were determined and listed in Table 2.

6.3. Day – by – day measurements:

The validity and the applicability of the proposed method and the reproducibility of the results obtained were studied by carrying out five replicate experiments at different concentrations of CPM in pure and pharmaceutical preparations. Using the above mentioned procedure, the absorbance of these concentrations was measured five times in the same day (intra-day) or daily for five days (inter-day) and the results were recorded to make statistical calculations. It was found that, the between day relative standard deviations are less than 3%, which indicated that the proposed methods were highly reproducible and successfully applied to determine CPM drug via the formation of ion pairs with BCP, ARS, ECR or CR reagents. The data were listed in Tables 4, 5.

**Table 4.** Inter- and intra-day precision for the determination of CPM drug in pure form using CR, ARS, ECR and BCP reagents.

| Reagent | Drug µg mL⁻¹ | Inter–day | Intra-day |
|---------|--------------|-----------|-----------|
|         | Found µg mL⁻¹ | Recovery (%) | RSD (%) | Found µg mL⁻¹ | Recovery (%) | RSD (%) |
| CR      | 10.00 | 9.960 | 99.60 | 1.11 | 10.06 | 100.6 | 0.54 |
|         | 40.00 | 40.26 | 100.70 | 1.07 | 40.39 | 101.0 | 0.39 |
|         | 120.0 | 118.0 | 98.33 | 0.30 | 118.0 | 98.33 | 0.73 |
| ARS     | 1.000 | 0.977 | 97.70 | 1.55 | 0.996 | 99.60 | 1.63 |
|         | 8.000 | 8.100 | 101.6 | 0.99 | 8.00 | 100.0 | 0.40 |
|         | 10.00 | 9.920 | 99.20 | 0.63 | 10.00 | 100.0 | 0.22 |
| ECR     | 16.00 | 16.12 | 100.8 | 1.24 | 16.00 | 100.0 | 0.67 |
|         | 60.00 | 60.12 | 100.2 | 0.55 | 60.40 | 100.7 | 0.11 |
|         | 120.0 | 120.4 | 100.3 | 0.40 | 120.3 | 100.3 | 0.36 |
| BCP     | 6.000 | 5.900 | 98.30 | 1.50 | 5.98 | 99.66 | 0.29 |
|         | 10.00 | 10.25 | 102.5 | 0.53 | 10.06 | 100.6 | 0.65 |
|         | 30.00 | 29.18 | 97.27 | 0.52 | 30.03 | 100.1 | 0.95 |

Number of replicates, n=5.
Table 5. Inter-day and intra-day precision for determination of CPM drug in Allergy tablets using CR, ARS, ECR and BCP reagents.

| Reagent | Drug µg mL⁻¹ | Inter-day | Intra-day |
|---------|--------------|-----------|-----------|
|         |              | Found µg mL⁻¹ | Recovery (%) | RSD (%) | Found µg mL⁻¹ | Recovery (%) | RSD (%) |
| CR      | 10.00        | 10.20     | 102.0     | 0.78    | 9.98       | 99.80     | 0.51    |
|         | 40.00        | 39.98     | 99.95     | 1.37    | 40.06      | 100.2     | 1.63    |
|         | 120.0        | 118.7     | 98.91     | 0.66    | 119.8      | 99.83     | 2.54    |
| ARS     | 1.000        | 1.007     | 100.7     | 2.45    | 0.990      | 99.00     | 2.54    |
|         | 8.000        | 8.040     | 100.5     | 1.90    | 8.10       | 101.3     | 0.99    |
|         | 10.00        | 9.800     | 98.0      | 0.75    | 10.09      | 100.9     | 1.10    |
| ECR     | 16.00        | 16.15     | 100.9     | 0.11    | 15.70      | 98.13     | 1.30    |
|         | 60.00        | 59.80     | 99.70     | 1.10    | 60.15      | 100.3     | 0.62    |
|         | 120.0        | 121.0     | 100.8     | 1.25    | 119.8      | 99.80     | 0.33    |
| BCP     | 6.000        | 6.010     | 100.2     | 1.64    | 5.91       | 98.50     | 0.36    |
|         | 10.00        | 9.990     | 99.90     | 0.21    | 9.89       | 98.90     | 1.37    |
|         | 30.00        | 30.18     | 100.6     | 0.93    | 29.60      | 98.70     | 0.48    |

Number of replicates, n=5.

6.4. Robustness

Robustness was examined by evaluating the effect of small variation in the method variables on its analytical performance. In these experiments, the absorbance of the formed ion pairs was determined by slight changes in optimum time while other conditions were remained unchanged. Similarly, the temperature of the reaction was slightly changed while the other conditions were kept unchanged and the absorbance of the formed ion pairs was measured. The recovery percentage was calculated in each case. It was found that the small variation in the method variables did not have great effect on the procedure as shown from the recovery values listed in Table 6. This indicates the reliability of the proposed methods during its routine application for the determination of CPM drug.

Table 6. Robustness parameter for the determination of CPM drug in using CR, ARS, ECR and BCP reagents.

| Reagent | Drug µg mL⁻¹ | Effect | Pure | tablet |
|---------|--------------|--------|------|--------|
|         |              |        | Found µg mL⁻¹ | Recovery (%) | RSD (%) | Found µg mL⁻¹ | Recovery (%) | RSD (%) |
| CR      | 100.0        | T(°C)  | 25   | 99.00 | 99.90 | 0.23 | 99.00 | 99.40 | 0.39 |
|         |              | t (min)| 5    | 99.70 | 100.0 | 0.26 | 99.30 | 99.80 | 0.22 |
| ARS     | 10.00        | T(°C)  | 25   | 10.00 | 99.90 | 0.26 | 99.30 | 99.80 | 0.22 |
|         |              | t (min)| 5    | 9.960 | 99.60 | 0.20 | 10.00 | 100.0 | 0.65 |
| ECR     | 60.00        | T(°C)  | 25   | 59.70 | 99.50 | 0.48 | 59.70 | 99.50 | 0.97 |
|         |              | t (min)| 3    | 59.70 | 99.50 | 0.60 | 59.80 | 99.60 | 0.60 |
| BCP     | 10.00        | T(°C)  | 10   | 10.00 | 100.0 | 2.46 | 9.90  | 99.00 | 1.50 |
|         |              | t (min)| 3    | 9.800 | 98.00 | 1.04 | 9.900 | 100.0 | 1.84 |
7. Application to Spiked Human Urine and Blood Serum

The proposed method was applied for the determination of CPM drug in spiked human urine and blood serum by following the general procedure described in the experimental part. The percentage recovery and standard deviation showed the non-interference of other materials present in urine or blood serum. The analytical results obtained for CPM drug determination in spiked human urine or blood serum samples were presented in Table 7.

Table 7. Application of the proposed method to CPM concentration measurements in spiked human urine and blood serum.

| Reagent | Drug taken µg mL⁻¹ | Found µg mL⁻¹ | Recovery% | RSD% |
|---------|---------------------|---------------|------------|------|
|         | Urine   | Blood | Urine   | Blood | Urine   | Blood |
| CR      | 10.00   | 9.800 | 10.15   | 98.00 | 101.5   | 0.99  |
|         | 40.00   | 40.50 | 39.80   | 101.3 | 99.50   | 0.59  |
|         | 120.0   | 119.2 | 118.8   | 99.30 | 99.00   | 0.42  |
| ARS     | 1.000   | 1.010 | 0.990   | 99.00 | 101.0   | 1.20  |
|         | 8.000   | 7.980 | 8.080   | 99.75 | 100.1   | 2.00  |
|         | 10.00   | 9.980 | 10.03   | 99.80 | 100.3   | 0.47  |
| ECR     | 16.00   | 16.10 | 16.08   | 100.6 | 100.5   | 0.93  |
|         | 60.00   | 59.50 | 60.20   | 99.20 | 100.3   | 1.40  |
|         | 120.0   | 120.2 | 119.5   | 99.50 | 100.3   | 0.66  |
| BCP     | 6.000   | 6.010 | 5.990   | 100.2 | 99.80   | 0.72  |
|         | 10.00   | 10.10 | 10.09   | 101.0 | 100.9   | 0.36  |
|         | 30.00   | 30.20 | 29.80   | 100.6 | 99.30   | 0.97  |

Intra-day

| Reagent | Drug taken µg mL⁻¹ | Found µg mL⁻¹ | Recovery% | RSD% |
|---------|---------------------|---------------|------------|------|
|         | Urine   | Blood | Urine   | Blood | Urine   | Blood |
| CR      | 10.00   | 9.92  | 9.97    | 99.20 | 99.70   | 0.55  |
|         | 40.00   | 40.30 | 59.80   | 100.8 | 99.70   | 0.35  |
|         | 120.0   | 120.4 | 120.5   | 100.3 | 100.4   | 0.69  |
| ARS     | 1.000   | 0.99  | 1.02    | 99.00 | 102.0   | 1.40  |
|         | 8.000   | 8.05  | 8.03    | 100.6 | 100.4   | 1.10  |
|         | 10.00   | 9.93  | 9.97    | 99.30 | 99.70   | 0.57  |
| ECR     | 16.00   | 16.15 | 15.90   | 100.9 | 99.40   | 0.67  |
|         | 60.00   | 60.10 | 58.90   | 100.2 | 98.10   | 1.10  |
|         | 120.0   | 118.8 | 118.5   | 99.00 | 98.80   | 2.20  |
| BCP     | 6.000   | 5.98  | 6.10    | 99.60 | 101.6   | 0.34  |
|         | 10.00   | 10.05 | 9.95    | 100.5 | 99.50   | 0.87  |
|         | 30.00   | 30.05 | 30.10   | 100.2 | 100.3   | 0.12  |

8. Conclusion

The dye reagents, BCP, ARS, ECR and CR have been utilized as ion pairing reagents for the determination of CPM drug in pure form, tablets, spiked human urine and blood serum samples. The proposed method was simple and no heating or long standing time was needed. The reagents used in the proposed method were cheaper, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The method was unaffected by slight varieties in experimental conditions such as time and reagent concentration. The proposed method gave results with good accuracy to allow determination of low concentration.
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