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Original Article

SPECTROPHOTOMETRIC DETERMINATION OF ARIPIPRAZOLE, CLOZAPINE AND SULPIRIDE BY ION- PAIR EXTRACTIONIN IN TABLETS AND BIOLOGICAL FLUIDS

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ABSTRACT

Objective: Simple, sensitive and extraction-free spectrophotometric methods have been developed for the determination of three antipsychotics drugs, namely aripiprazole (ARP), clozapine (CLP) and sulpiride (SUP) both in tablets and in biological fluids.

Methods: Two spectrophotometric methods are based on the formation of yellow colored ion-pair complexes between the studied drugs and two sulphonphthalein acid dyes, bromophenol blue (BPB) and bromothymol blue (BTB) with absorption maxima at 408 and 406 nm, respectively.

Results: Molar ratio of formed ion-pair complex was found to be 1:1 as deduced by Job's method. Several parameters such as pH and buffer type, reagent volume, sequence of addition and effect of extracting solvent were optimized to achieve high sensitivity, stability, low blank reading and reproducible results. Various analytical parameters have been evaluated and the results have been validated by statistical data. A proposal for the reaction pathway was postulated.

Conclusion: The proposed methods were successfully applied to the analysis of commercial tablets containing the drugs and the results were in good agreement with those obtained with reported methods. The proposed methods were further applied to the determination of the studied drugs in spiked human serum and urine.

Keywords: Aripiprazole, Clozapine, Sulpiride, Ion-pair complex, Bromophenol blue, Bromothymol blue.

INTRODUCTION

Aripiprazole (ARP), clozapine (CLP) and sulpiride (SUP) are structurally related atypical antipsychotics. They are used in the treatment of schizophrenia and other psychotic syndromes. It is reported that they are effective in the treatment of both positive and negative symptoms of schizophrenia, and that they are less likely to produce extra pyramidal side effects when compared with classical antipsychotics. The advantages of the therapeutic profile of the three drugs have led to increasing use of them in treatment of schizophrenic patients [1, 2].

However, high doses of these atypical antipsychotics are suspected to pose an increased risk for extra pyramidal side effects or other side effects [1, 3–7]. Aripiprazole (ARP) (Fig. 1a), chemically 7-[4-[4-(2, 3-dichlorophenyl) piperazine -1- yl] butoxy] - 3, 4-dihydro-1H-quinolin-2-one. A survey of pertinent literature revealed that few analytical methods reported for determination of aripiprazole in pharmaceutical dosage forms and biological samples include chromatographic [8–16] and spectrophotometric [17, 18] methods.

Clozapine (CLP) (Fig. 1b), (8-chloro-11-(4-methyl-piperazin-1-yl)-5H-dibenzo[b,e] [1,4]-diazepine; is an atypical antipsychotic drug. Different methods for the analysis of clozapine have been reviewed. These methods include high performance liquid chromatography [19–22], liquid chromatography [23, 24], gas chromatography [25], capillary zone electrophoresis [26, 27] and linear scan voltammetry [28–30]. Few photometric [31, 32] and fluorimetric [33] methods have been reported for the analysis of clozapine. Sulpiride (SUP) (Fig. 1c), 5-(Aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxy-benzamide. A review of the literature revealed that several analytical methods have been described for the determination of sulpiride in pharmaceuticals or biological fluids, including spectrophotometric [34–37], fluorimetric [38], chromatographic [39–44], electrophoretic [45-47], voltammetric [48] and chemiluminometric [49]; however, the methods proposed for the analysis of biological fluids suffer the inconvenience of time-consuming procedures and expensive instrumentation.

Most of the reported methods (except spectrophotometric methods) are either not appropriately sensitive or tedious and utilized expensive instruments that are not available in most quality control laboratories and the procedures are not simple to perform. Therefore the aim of the present work is to develop a simple, accurate, sensitive and low-cost spectrophotometric method for the quantification of three antipsychotic drugs, namely aripiprazole, clozapine and sulpiride. The proposed methods are based on the ability of the studied drugs to form stable ion-pair complexes with BPB and BTB.

MATERIALS AND METHODS

Apparatus

All the absorbance spectral measurements were made using spectroscan 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.

i. A 1×10^{-3} M of bromophenol blue and bromothymol blue (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.

ii. 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 were prepared by standard methods.

iii. Pharmaceutical grade of ARP, CLP and SUP 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 ARP, CLP and SUP were prepared separately by dissolving accurately weighed 10 mg of each drug in 1.0 ml of concentrated sulphuric acid and finally the volume was made up to 100 ml with distilled water (100 µg/ml).

General recommended procedures

Procedures for calibration curves

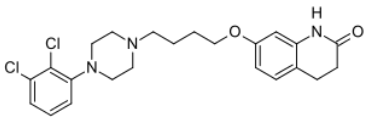
Into a series of separated funnels, accurately measured aliquots of ARP, CLP and SUP, in the concentration range shown in (Table 1) were pitted out. A volume of 2.0 ml of 5×10^{-3} M BPB or BTB was added. Then, 2.0 ml of the optimum pH value for each system as recorded in table 1was added and the volume was completed to 10 ml with distilled water. The ion-pairs were extracted with 10 ml of dichloromethane by shaking for 2.0 min and then, the combined dichloromethane 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 an addition of drugs. In both the methods, a standard curve was prepared by plotting the increasing absorbance values versus concentrations of drug. A linear equation for the standard curve was calculated by linear regression.

Procedure for tablets

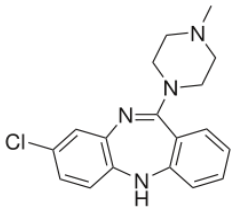
At least ten tablets of the drugs were weighted into a small dish, powdered and mixed well. A portion equivalent to 10 mg of ARP, CLP and SUP was weighted and dissolved in distilled water with 1.0 ml of concentrated sulphuric acid, filtered into a 100 ml calibrated flask and diluted to volume with water. Solutions of working range concentration were prepared by proper dilution of this stock solution with water and followed the above procedure for the calibration curve.

Procedures for human serum and urine

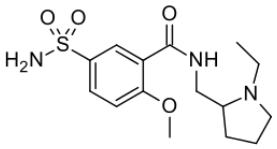
The proposed methods were applied to the determination of the studied drugs in spiked urine and serum provided from several healthy volunteers. Spiked urine was 50-fold diluted with distilled water. A 10 ml of serum sample was deproteinzed by adding 5 ml of acetonitrile in a centrifuge for 5 min at 1000 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.



(a) ARP



(b) CLP



(c) SUP

Fig. 1: Chemical structure of the studied drugs.

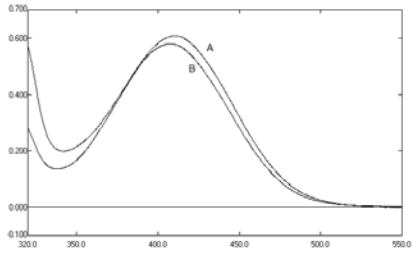


Fig. 2: Absorption spectra of CLP (6 µg/ml) with dyestuff: A-bromophenol blue, *B*-bromthymol blue

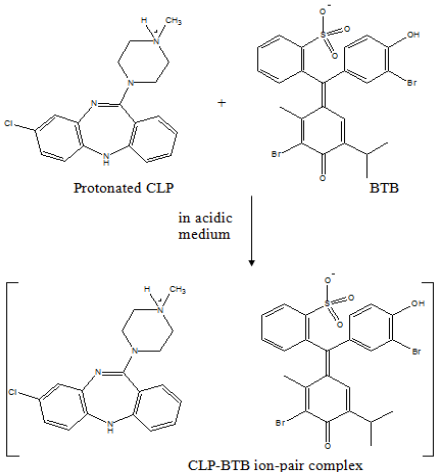


Fig. 3: Proposed reaction pathway for the formation of ion-pair complex between CLP and BTB measured spectrophotometrically at 406 nm

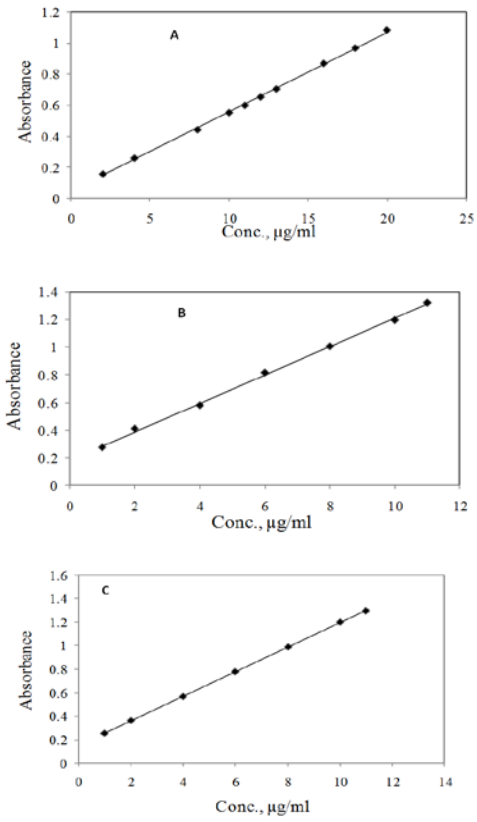


Fig. 4: Calibration curves for determination of a-ARP (2–20µg/ml), b-CLP (1–11µg/ml) and c-SUP (1–11µg/ml) using BPB.

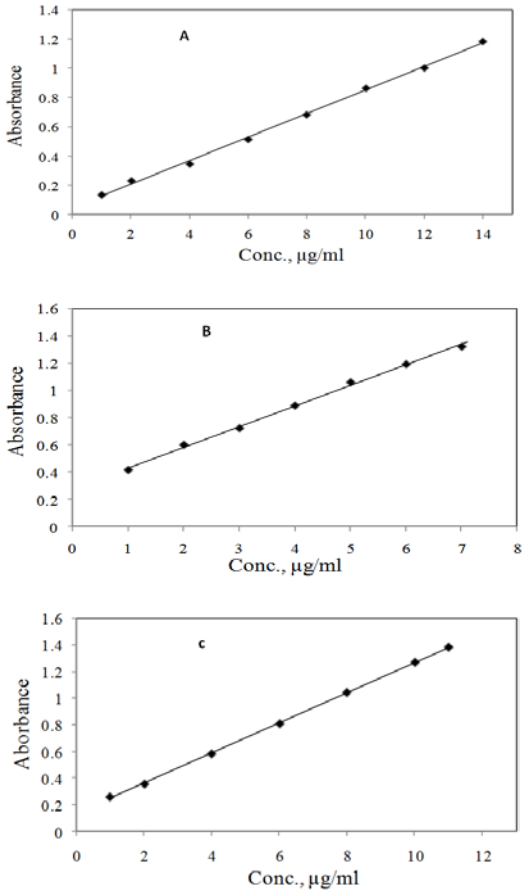


Fig. 5: Calibration curves for determination of a-ARP (1–14µg/ml), b-CLP (1–7µg/ml) and c-SUP (1–11µg/ml) using BTB

RESULTS AND DISCUSSION

Spectral characteristics

Absorption spectra of the yellow color of CLP–BPB and BTB ion-pair complex with a maximum absorbance (λ_{max}) at 408 and 406 nm, respectively is shown in (Fig. 2). The colorless blanks have practically negligible absorbance.

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.

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 NaOAc_HCl buffer. It is evident that the absorbance of ion pair complex with BPB was found to be maximal at pH 3, while with BTB the absorbance was maximal at pH 3 for ARP and pH 4.4 for both CLP and SUP. Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5-4.0 ml). The higher absorbance value obtained at using 2.0 ml of buffer solutions.

Effect of reagent concentration

The ARP, CLP and SUP concentrations were kept constant, while the concentrations of BPB or BTB was varied from 0.5–4.0 ml of 5×10^{-3}M. The results showed that the absorbance of the extracted ion-pairs increased by increasing the BPB or BTB concentrations till 2.0 ml. After this volume, the absorbance remains constant by increasing the volume of the reagents. So an excess of reagents has no effect on the determination of the drugs.

Table 1: Analytical parameters and optical characteristics of the studied drugs

Parameters	BPB			BTB		
	ARP	CLP	SUP	ARP	CLP	SUP
λ_{max} (nm)	408	408	408	406	406	406
pH	3	3	3	3	4.4	4.4
Beer's law limit, µg/ml	2–20	1–11	1–11	1–14	1–7	1–11
Molar absorptivity, l mol^{-1} cm^{-1}	2.4×10^4	3.9×10^4	4.01×10^4	4.10×10^4	6.17×10^4	4.30×10^5
Sandell's sensitivity, Ng/cm^2	10.8	8.3	8.5	10.7	5.2	7.9
Correlation coefficient (r)	0.9996	0.9992	0.9999	0.9991	0.9993	0.9998
Linear regression equation*						
Intercept (a)	0.0641	0.1680	0.1440	0.0410	0.2511	0.1371
Slope (b)	0.0506	0.1030	0.1058	0.0839	0.1587	0.1128
$S_{y/x}$	0.0082	0.0159	0.0037	0.0179	0.0127	0.0071
S. D. of slope (S_b)	6.7×10^{-3}	2×10^{-3}	4.8×10^{-3}	1.8×10^{-3}	2.9×10^{-3}	9.1×10^{-3}
S. D. of intercept (S_a)	0.020	0.025	0.006	0.029	0.024	0.011
LOD, µg/ml	0.2503	0.1230	0.1212	0.1359	0.0813	0.1134
LOQ, µg/ml	0.8338	0.4098	0.4036	0.4528	0.2714	0.3778

*A= a+ b C, where A is the absorbance and C is the concentration of drug in µg/ml.

Table 2: Evaluation of day accuracy and precision of the proposed methods

Method	Drugs	Drug taken, µg/ml	Drug found, µg/ml	Recovery[a], %	RSD[b], %	RE, %
		1.6	1.59	100.002	0.420	-0.625
BPB	ARP	4.0	3.99	99.999	0.207	-0.250
		8.0	7.9	99.998	0.172	-1.250
		0.8	0.79	99.999	0.428	-1.250
	CLP	1.6	1.59	99.991	0.264	-0.625
		2.4	2.39	99.999	0.384	-0.416

		0.8	0.79	98.999	0.264	-1.250
	SUP	2.4	2.39	99.998	0.351	-0.416
		3.2	3.19	99.989	0.493	-0.312
		0.8	0.799	99.996	0.351	-0.125
BTB	ARP	3.2	3.199	99.999	0.405	-0.031
		1.0	0.999	99.991	0.258	-0.100
		0.8	0.799	99.999	0.496	-0.125
	CLP	1.6	1.599	99.999	0.237	-0.062
		2.4	2.399	99.997	0.364	-0.041
		0.8	0.799	99.999	0.799	-0.125
	SUP	2.4	2.401	100.002	0.223	0.041
		4.0	3.991	99.999	0.141	-0.225

[a]Mean value of five determinations, [b]RE: Relative error.

Table 3: Recovery of the studied drugs in pharmaceutical formulations using the proposed methods

Method	Drugs	Drug taken, µg/ml	Drug formulation	Drug found, µg/ml	Recovery[a], %	RSD, %	RE[b], %
	ARP	1.6		1.599	99.999	1.028	-0.062
BPB		4.0	Aripiprex	3.999	99.997	0.544	-0.025
		8.0	10mg/tablet	7.999	100.009	0.326	-0.012
	CLP	0.8	Clozapex,	0.801	100.001	0.844	0.125
		1.6	100mg/tablet	1.599	99.999	0.821	-0.062
		2.4		2.399	97.999	0.781	-0.041
	SUP	0.8	Dogmatil,	0.799	99.992	0.524	-0.125
		2.4	200mg/tablet	2.399	101.010	0.906	-0.041
		3.2		3.199	99.999	0.993	-0.031
	ARP	0.8		0.799	99.997	0.975	-0.125
BTB		3.2	Aripiprex	3.199	99.989	0.854	-0.031
		4.0	10mg/tablet	3.999	101.074	0.749	-0.025
	CLP	0.8	Clozapex,	0.799	99.999	0.834	-0.125
		1.6	100mg/tablet	1.599	100.256	0.473	-0.062
		2.4		2.399	99.998	0.187	-0.041
	SUP	0.8	Dogmatil,	0.799	101.003	0.830	-0.125
		2.4	200mg/tablet	2.399	99. 981	1.063	-0.041
		3.2		3.201	100.002	0.993	0.031

[a]Mean value of five determinations, [b]RE: Relative error.

Table 4: Recovery of the studied drugs from spiked human urine

Method	Drugs	Drug added, µg/ml	Drug found, µg/ml	Recovery [a], %	RSD, %	RE[b], %
		1.6	1.599	99.999	1.046	-0.062
BPB	ARP	2.0	1.999	100.010	0.857	-0.050
		1.0	1.001	98.996	0.399	0.100
		0.8	0.799	101.209	0.525	-0.125
	CLP	1.6	1.599	99.994	1.097	-0.062
		2.4	2.399	102.341	1.030	-0.041
	SUP	0.8	0.799	99.999	1.056	-0.125
		1.6	1.599	99.993	1.021	-0.062
		4.0	3.990	101.008	0.615	-0.250
		0.8	0.799	99.999	0.726	-0.125
BTB	ARP	3.2	3.199	98.750	0.416	-0.031
		4.0	3.999	100.896	1.052	-0.025
		0.8	0.799	102.700	0.958	-0.125
	CLP	1.6	1.601	100.001	0.733	0.062
		2.4	2.399	99.999	0.992	-0.041
	SUP	0.8	0.799	100.003	0.976	-0.125
		2.4	2.399	99.996	0.726	-0.041
		3.2	3.199	99.991	0.761	-0.031

[a]Mean value of five determinations, [b]RE: Relative error.

Choice of organic solvents

Different organic solvents as dichloromethane, carbon tetrachloride, chloroform and ether were tested as extractive solvents for the proposed method. Dichloromethane was preferred to other solvents for its selective and obtained the highest absorbance with dichloromethane.

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.

Shaking time of 0.5-5 min provided constant absorbance and hence, 2.0 min was selected as the optimum shaking time.

Reaction mechanism

Clozapine forms ion-pair complex with BPB or BTB dye, since it contains the tertiary amino group which is protonated. In the ring of 1H-[1, 4] diazepine, protonation is very difficult due to resonance and steric effects.

Therefore, the only site in CLP vulnerable for protonation is the nitrogen bonded to electron donating methyl group in the piperazine ring [50] and finally the protonated CLP forms ion-pair with the BPB or BTB dye. The suggested reaction pathway for the reaction product of CLP - BTB ion–pair complex formation for example, is given in Fig. 3.

Table 5: Recovery of the studied drugs from spiked human serum

Method	Drugs	Drug added, µg/ml	Drug found, µg/ml	Recovery [a], %	RSD, %	RE[b], %
		1.6	1.599	99.999	1.046	-0.062
BPB	ARP	4.0	4.001	100.003	0.857	0.025
		8.0	7.999	101.023	0.399	-0.125
		0.8	0.799	102.198	0.525	-0.125
	CLP	1.6	1.599	99.999	1.097	-0.062
		2.4	2.399	100.006	1.030	-0.041
	SUP	0.8	0.799	99.991	1.056	-0.125
		2.4	2.401	99.999	1.021	0.041
		3.2	3.199	99.986	0.615	-0.031
		0.8	0.799	99.994	1.173	-0.125
BTB	ARP	3.2	3.199	100.488	1.098	-0.031
		4.0	3.999	98.986	0.996	-0.025
		0.8	0.799	101.023	1.002	-0.125
	CLP	1.6	1.599	102.344	0.886	-0.062
		2.4	2.399	100.010	0.344	-0.041
	SUP	0.8	0.799	99.816	0.548	-0.125
		2.4	2.399	99.999	0.829	-0.041
		3.2	3.201	102.001	0.790	0.031

[a]Mean value of five determinations, [b]RE: Relative error.

Effect of temperature on the colored complexes

The effect of temperature on colored complexes was studied over the range 20-35 °C. It was found that the absorbance of the ion pair complex was constant up to 30 °C. At higher temperatures, the drug concentration was found to increase due to the volatile nature of the dichloromethane. Therefore, the temperature chosen was 30 °C as the best temperature for micro-determination of the drugs under study in pure and pharmaceutical formulations.

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 was obtained when extracted after 1.5 min shaking. Therefore, shaking time of 2.0 min was maintained throughout the experiment.

Composition of the ion-pair complexes

In order to establish the molar ratio between ARP, CLP and SUP on one side and BPB or BTB reagent used on the other, Job's method of continuous variation was applied [51]. In this method, 5×10^{-3} M solutions of drugs and reagent were 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. 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:

$$D^+_{(aq)} + R^-_{(aq)} \leftrightarrow D^+R^-_{(aq)} \leftrightarrow D^+R^-_{(org)}$$

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.

Quantification

Under the optimum conditions described above, the calibration graphs were constructed for the investigated drugs. The molar absorptivity, Sandells sensitivity, concentration range, regression equation and correlation coefficient for each drug are tabulated in (Table 1). A linear relationship was found between the absorbance at λ_{max}. Regression analysis of Beer's law plotted at λ_{max} reveals a good correlation (Figs. 4, 5). The graphs showed a negligible intercept, which was calculated by the least-squares method's regression equation, A = a + bC (where A is the absorbance of 1.0 cm layer, b is the slope, a is the intercept and C is the concentration of the measured solution in µg/ml. The high molar absorptivities of the resulting colored complexes indicated high sensitivity of the method (2.40×10^4 – 4.30×10^5). The SUP - BTB method was found to be the most sensitive of all these methods with high ε value. The limit of detection (LOD) and limit of quantitation (LOQ) are calculated according to ICH guidelines [53]. The results are as shown in (Table 1).

Accuracy and precision

In order to determine the accuracy and precision of the recommended procedure five replicate determinations at three different concentrations of the studied drugs were carried out. 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).

Analysis of dosage forms

To evaluate the validity and reproducibility of the methods, known amounts of the

ARP, CLP and SUP 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 3. 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 method, also allowed the in vitro determination of ARP, CLP and SUP in spiked human serum and urine samples. Thus the proposed method is sufficient for routine estimation of the drugs in human serum and urine. A prior extraction step by the same organic solvent was adopted before application of the method. The results obtained are satisfactorily accurate and precise (Tables 4, 5).

CONCLUSION

A significant advantage of the extractive spectrophotometric methods is that it can be applied for the determination of individual compounds in a multicomponent mixture. The proposed methods make use of 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. Moreover, the methods are accurate, reproducible, adequately sensitive and free from interference caused by the excipients expected to be present in tablets. The methods were successfully applied to the spiked human serum and urine.

CONFLICT OF INTERESTS

Declared None

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