Research Article

Spectrophotometric Determination of Four Selected Antipsychotic Drugs in Dosage Forms and Biological Fluids

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ABSTRACT

A simple, rapid and sensitive extractive Spectrophotometric method has been developed for the determination of four antipsychotic drugs, namely Aripiprazole (ARZ), Clozapine (CLZ), Olanzapine (OLZ) and Sulpiride (SLP) both in pharmaceutical formulations and in spiked human serum and spiked human urine. The method is based on the formation of yellow colored ion-pair complexes between the studied drugs and methyl orange (MO) with absorption maximum at 428 nm. The stoichiometry of the complex in either case was found to be 1: 1 and the conditional stability constant (K_F) of the complexes has also been calculated. Reaction conditions were optimized to obtain the maximum color intensity. Beer's law was obeyed in the concentration ranges of 2-14, 2-14, 2-14 and 2-22 µg/ml with ARZ, CLZ, OLZ and SLP, respectively. The Sandell's sensitivity is found to be 4.483, 4.075, 4.047 and 7.471 ng cm^{-2} for ARZ, CLZ, OLZ and SLP, respectively. Various analytical parameters have been evaluated and the results have been validated by statistical data. The proposed method was 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 method was further applied to the determination of the studied drugs in spiked human serum and urine. A proposal for the reaction pathway was postulated.

Keywords: Aripiprazole, Clozapine, Ion-pair complex, Methyl orange, Olanzapine, Sulpiride.

INTRODUCTION

Schizophrenia is a severe psychiatric disorder affecting approximately 1.5 % of the world's population, for which antipsychotic (AP) drugs are the treatment of choice. Indeed, AP medications are one of the fastest growing products in the pharmaceutical industry.[1] Clozapine, Olanzapine, Aripiprazole and Sulpiride 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 four drugs have led to increasing use of them in treatment of schizophrenic patients.[2,3] However, high dose of these atypical antipsychotics are suspected to pose an increased risk for extra pyramidal side effects or other side effects.[2, 4-7]

The chemical name of Aripiprazole (ARZ) is 7-(4-(4-2,3-dichlorophenyl) -1-piperazinyl) butoxy)- 3,4 dihydrocarbostyril. The structural formula of Aripiprazole is shown in (Scheme 1a). A survey of pertinent literature revealed that few analytical methods reported for determination of Aripiprazole in pharmaceutical dosage forms and biological samples include HPLC[8-11], gas-chromatography-mass spectrometry (GC-MS)[12], liquid chromatography-tandem mass spectrometry (LC-MS/MS)[13-17], capillary electrophoresis[18,19] and Spectrophotometric methods[20-23] have been described for the determination of APZ in pharmaceutical preparations.

Clozapine (CLZ), chemically known as 8-chloro-11- (4-methyl- l-piperazinyl)-5H-dibenzo[b,e] [1,4] diazepine (Scheme 1b). The drug is not official in any pharmacopoeia. Different methods for the analysis of clozapine have been reviewed. These methods include liquidchromatography[24-27], high performance liquid chromatography[28-32], gas chromatography[33], capillary zone electrophoresis[34, 35], linear scan voltammetry[36-38] and Spectrophotometric.[39] Most of these methods suffer from some drawbacks. HPLC and the other methods utilized expensive instruments that are not available in most quality control laboratories, and the procedures are not simple to perform.

Olanzapine (OLZ), 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]-benzodiazepine (Scheme 1c). A facile, selective and sensitive method for OLZ determination is of importance in routine analysis. Various methods have been reported for its assay in pharmaceuticals and in biological samples include GC[40], HPLC[41,42], UV spectrophotometry[43,44] and FIA.[45] A few visible Spectrophotometric methods[46-48] have been reported for its analysis.

Sulpiride (SLP), 5-(Aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-methoxy-benzamide, (Scheme 1d). A number of analytical methods have been published in the literature for the analysis of Sulpiride in pharmaceutical formulations and biological fluids. These methods include spectrofluorimetry[49-51], derivative synchronous fluorescence[52,53], spectrophotometry[54,55], gas chromatography (GC)[56], high-performance liquid

chromatography (HPLC)[57-60], capillary electrophoresis[61, 62] and adsorptive stripping voltammetry.[63]

The reported methods (except Spectrophotometric methods) are either not appropriately sensitive or tedious and need expensive, sophisticated and dedicated instrumentation. Therefore the aim of the current work is to develop a simple, accurate, sensitive, low-cost and proficient Spectrophotometric method for the quantification of four antipsychotics drugs, namely Aripiprazole, Clozapine, Olanzapine and Sulpiride. The proposed method is based on the ability of the studied drugs to form ion associations with MO. The reaction conditions and the application of the method to the determination of the studied drugs in tablets and in biological fluids have been established.

Experimental

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.

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.

A 5×10^{-3} M of methyl orange was prepared by dissolving the accurate weighed amount of 20 mg in 100 ml water. 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.

Pharmaceutical grade of ARZ, CLZ, OLZ and SLP certified to be 99.85% pure was obtained as gift were kindly supplied from Egyptian International Pharmaceutical Industries Company (EIPICo), Egypt. Stock solutions of pure ARZ, CLZ, OLZ and SLP were prepared separately by dissolving 10 mg (accurately weighed) 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

Procedure for calibration curve

Into a series of separated funnels, accurately measured aliquots of ARZ, CLZ, OLZ and SLP in the concentration range shown in (Table 1) were pitted out. A volume of 2.5 ml of 5×10^{-3} M MO (for ARZ, CLZ, OLZ), and 6.0 ml (for SLP) were added. Then, 1.0 ml of acetate buffer solution (pH 3.4 for ARZ and CLZ and pH 4.2 for OLZ and SLP) were 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 yellow colored ion-pair complexes were measured within 20 min of extraction at 428 nm against reagent blank prepared in the same manner except addition of drugs.

Procedure for tablets

At least ten tablets of the drugs were weight into a small dish, powdered and mixed well. A portion equivalent to 10 mg of ARZ, CLZ, OLZ and SLP were weight 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 calibration curve.

Procedure for human serum and urine

Human serum samples were thawed at room temperature and mixed well. A 4.0 ml aliquot of serum was added and mixed briefly with 4.0 ml of acetonitrile. After centrifugation at approximately 3000 rpm for 3.0 min, filtration then dilution into 100 ml in calibrated flask was done. Aliquots of 5.0 ml of each serum sample spiked with different concentrations levels of drugs, 0.5 ml of 1.0 M sodium hydroxide, was added and mixed briefly, samples were extracted by addition of 10 ml of dichloromethane followed by rotation for approximately 2.0 min. After centrifugation at approximately 3000 rpm for 10 min, the organic layer was quantitatively transferred to a separating funnel, then proceeds as described for calibration curve. Human urine samples were thawed at room temperature and mixed well, centrifugation at approximately 3000 rpm for 3 min followed by filtration process. A 5.0 ml aliquot of each sample was placed into a screw cap culture tube then spiked with different concentrations levels of drugs, the medium turned to alkaline with 0.5 ml of 1.0 M sodium hydroxide. Samples were extracted by addition of 10 ml of dichloromethane followed by rotation for approximately 2.0 min. After centrifugation at approximately 3000 rpm for 10 min; the organic layer was quantitatively transferred to a separating funnel, then proceeds as described for calibration curve.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of the ion-pair complexes were measured in the range 400-600 nm against dichloromethane (blank). Antipsychotics cations were found to react with MO dye anions in acidic buffer and gave an intense color with a maximum absorption at 428 nm (Figure 1). Therefore, all the following measurements are carried out at 428 nm against blank. The effects of the reagent concentrations, mixing time, sequence of addition with respect to maximum sensitivity, minimum blank adherence to Beer's law and stability were studied through control experiments. The optimum conditions were established by varying one variable and observing its effect on the absorbance of the colored product.

Effect of 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 was noticed that the maximum color intensity and constant absorbances were observed in NaOAc–AcOH buffer of (pH 3.4 for ARZ and CLZ and pH 4.2 for OLZ and SLP) (Figure 2). 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 dye concentration

Keeping other conditions unaltered, the effect of 5×10^{-3} M MO dye concentration on the absorbance was investigated. The results appeared that the maximum absorbance was at using 2.0 ml of MO dye for ARZ, CLZ and OLZ but 6.0 ml of MO dye used with SLP. Excess of MO dye did not have any effect either on the color of the ion-pair complexes or on the absorbance.

Choice of organic solvents

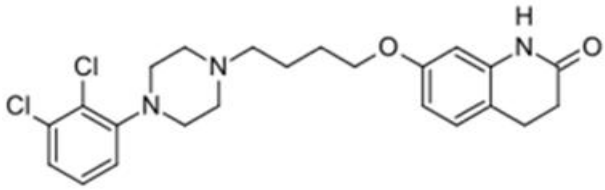
Different organic solvents as dichloromethane, carbon tetrachloride, chloroform and ether were tested as extractive solvents for the proposed method. Dichloromethane was preferred to other solvents for its selective and obtained highest absorbance with dichloromethane. It was also observed that only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

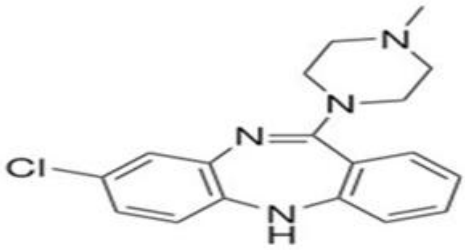
Clozapine forms ion-pair complexes with MO dye, since it contains tertiary amino group which is protonated. In the ring of 1H-[1, 4] diazepine, protonation is very difficult due to resonance and steric effects. Therefore, the only site in CLZ vulnerable for protonation is the nitrogen bonded to electron donating methyl group in the piperazine ring[64] and finally the protonated CLZ forms ion-pair with the MO dye. The suggested mechanism for the reaction product of CLZ - MO ion–pair complex formation for example, is given in Scheme 2.

Composition of the ion-pair complexes

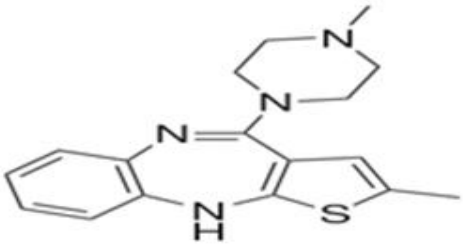
The composition of the ion-pair complexes formed between ARZ, CLZ, OLZ and SLP drugs and the MO dye were determined by Job's continuous variation method[65]. In this method, a series of solutions were prepared in which the total volume of the drugs and reagent was kept at 2.0 ml and the procedures were completed as described under general procedures and calibration graphs. The absorbance of each solution was plotted against the mole fraction of drug. The plot reached a maximum value at a mole fraction of 0.5 (Figure 3), which indicated that a 1: 1 (drug: dye) ion-pair are formed through the electrostatic attraction between positive protonated drugs and methyl orange anions.



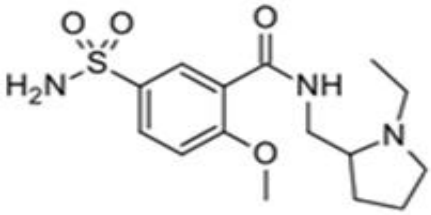
A. Aripiprazole



B. Clozapine



C. Olanzapine



D. Sulpiride

Scheme 1: Chemical structure of the studied drugs

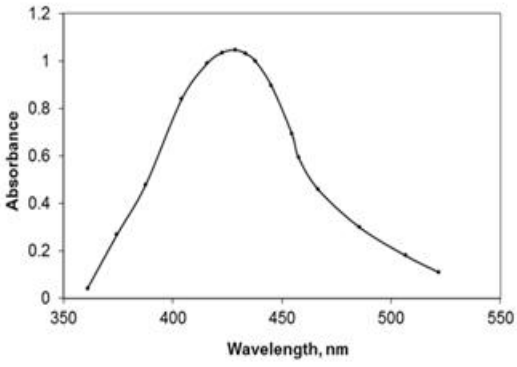


Figure 1: Absorption spectra of CLZ–MO ion-pair complex (concentration of CLZ was 10 µg/ml).

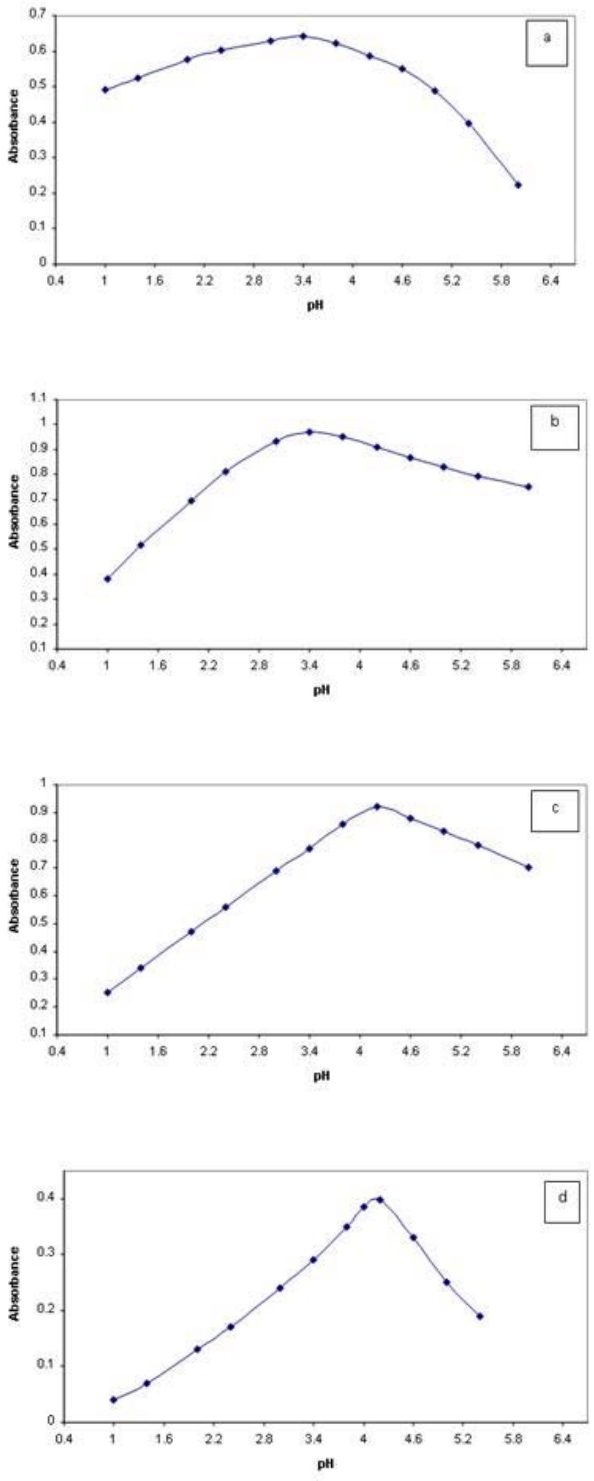
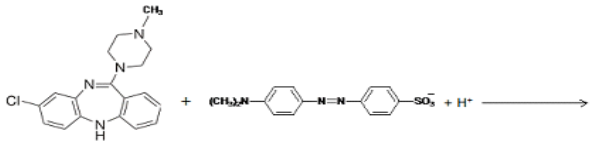


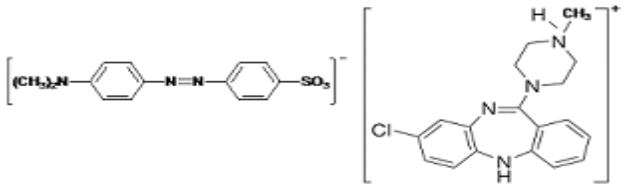
Figure 2: Effect of pH on the absorbance of ion-pair complex:(a) ARZ, (b) CLZ, (c) OLZ and (d) SLP using 5×10^{-3} M of MO.

Effect of temperature on the colored complexes

The effect of temperature on colored complexes was investigated by measuring the absorbance values at different temperatures. It was found that the colored complexes were stable up to 35°C. At higher temperatures, the drug concentration was found to increase due to volatile nature of the dichloromethane. As a result, the absorbances of the colored complexes increased. However, the complexes were stable for at least 24 h without any change in color intensity or in λ_{max} at room temperature.

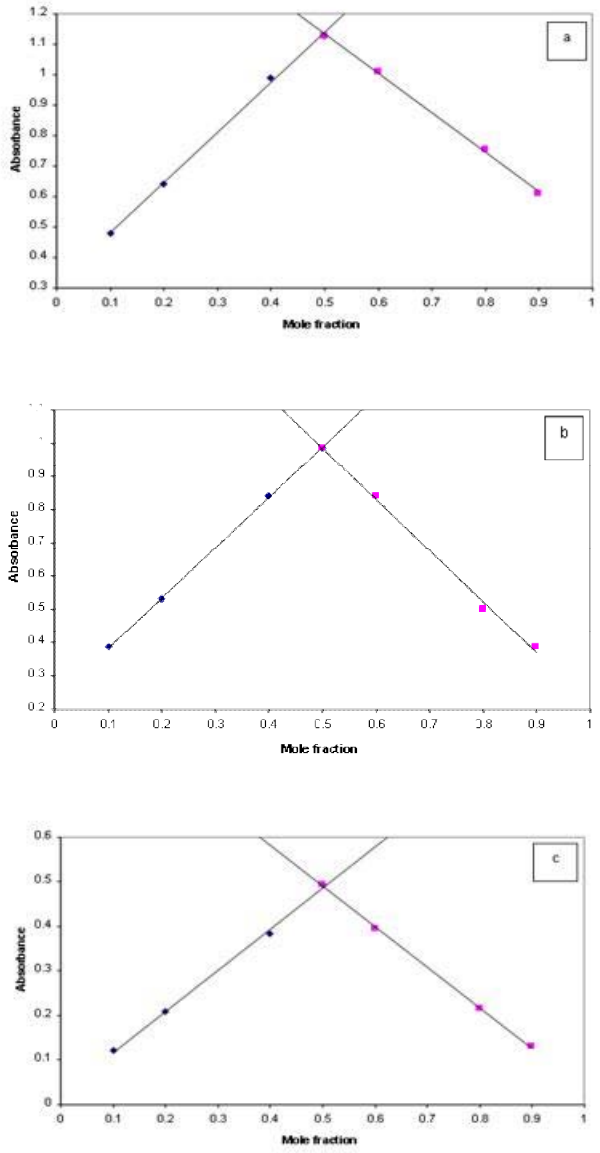


CLZ MO



CLZ-MO ion-pair complex

Scheme 2: The possible reaction mechanism for the formation of ion-pair complex CLZ with MO



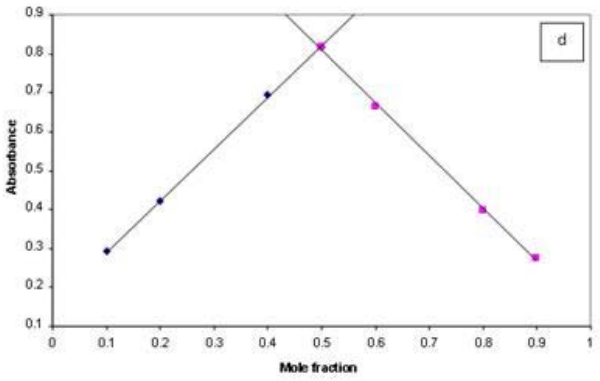


Figure 3: Job's method of continuous variations of MO with (a) ARZ, (b) CLZ, (c) OLZ and (d) SLP.

Conditional stability constants (K_f)

The conditional stability constants (K_f) of the ion-pair complexes were calculated from continuous variation data using the following formula;[66]

$$K_f = \frac{A/A_m}{[1 - A/A_m]^{n+1} C_D^n}$$

Where A and A_m are the observed maximum absorbance and the absorbance value of all the drugs present is associated, respectively. C_D is the molar concentration corresponding to the maximum in absorbance and n is the stoichiometric constant with which dye ion associates with drugs. The log K_f values for ARZ, CLZ, OLZ or SLP ion-pair complexes were 5.768, 5.601, 5.197 or 4.876, respectively.

Table 1: Optical characteristics and statistical data of the regression equations of the proposed method

Parameters	ARZ	CLZ	OLZ	SLP
pH	3.4	3.4	4.2	4.2
Volume of MO dye (ml)	2.5	2.5	2.5	6.0
Beer's law limit, µg/ml	2 - 14	2 - 14	2 - 14	2 - 22
Stability constant	5.768	5.601	5.197	4.876
Molar absorptivity, L mol^{-1} cm^{-1}	10.3×10^4	8.02×10^4	7.72×10^4	4.57×10^4
Sandell's sensitivity, ng cm^{-2}	4.483	4.075	4.047	7.471
Correlation coefficient (r)	0.9998	0.9997	0.9998	0.9998
Linear regression equation*				
$S_{y/x}$	7.61×10^{-3}	4.65×10^{-3}	5.70×10^{-3}	5.27×10^{-3}
Intercept (a)	0.0815	0.1950	0.1420	0.1943
Slope (b)	0.0884	0.0862	0.0827	0.0446
S.D. of slope (S_b)	9.18×10^{-4}	7.36×10^{-4}	9.01×10^{-4}	3.93×10^{-4}
S.D. of intercept (S_a)	0.0147	0.0131	0.0004	0.0013
LOD, µg/ml	0.0716	0.0734	0.0765	0.1419
LOQ, µg/ml	0.2384	0.2445	0.2548	0.4726

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

Table 2: Evaluation of accuracy and precision of the proposed method

Drugs	Drug taken, µg/ml	Drug found, µg/ml	Recovery[a], %	RSD, %	RE[b], %
ARZ	4	3.99	99.99	0.828	-0.250
	10	9.99	99.99	0.693	-0.100
	12	11.9	99.74	0.229	-0.833
CLZ	4	3.99	99.99	0.542	-0.250
	6	5.99	99.99	0.258	-0.166
	10	9.99	99.99	0.519	-0.100
OLZ	4	3.99	99.99	1.463	-0.250
	8	7.99	99.99	0.827	-0.125
	10	9.99	99.99	1.027	-0.100
SLP	8	7.99	99.99	0.342	-0.125
	10	9.99	99.99	0.510	-0.100
	12	11.9	99.99	0.395	-0.833

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

Table 3: Application of the proposed method for the analysis of the studied drugs in pharmaceutical formulations

Drugs	Tablet brand name	Labeled mg content	Found, mg	Recovery[a], %	t- and F-test[b]	Reported methods
ARZ	Aripiprex[1],	10	9.89	99.30±0.18	t=2.253 F=1.671	101.62±0.16
CLZ	Clozapex[2],	100	99.89	98.41±0.32	t=1.691 F=2.524	98.21±0.25
OLZ	Olazine[3],	10	9.94	98.92±0.45	t=0.675 F=1.823	98.54±0.68
SLP	Dogmatil[4],	200	199.6	97.76±0.89	t=1.442 F=1.774	96.01±1.47

Produced by: [1] S.P.I.for Al Andalous Medical Company, 6th of October City, Egypt; [2]APEX Pharma S.A.E.,10th of Ramadan City, Egypt; [3]EIPICo, 10th of Ramadan City, Egypt; [4]Sanofi- Aventis, S.A.E., El-Ameryia-Zeitoun, Egypt; [a]Mean value of five determinations; [b]Theoretical value for t- and F-values for five degrees of freedom and 95% confidence limits are 2.57 and 5.05, respectively.

Table 4: Application of the proposed method for the analysis of the studied drugs in spiked human serum

Drugs	Drug taken µg/ml	Drug found, µg/ml	Recovery[a], %	RSD, %	RE[b], %
ARZ	4	4.05	103.2	3.805	1.250
	8	8.13	104.1	4.816	1.625
	10	10.12	103.1	3.680	1.200
CLZ	4	3.93	96.93	4.710	-1.750
	6	5.93	97.22	3.209	-1.166
	10	9.87	96.77	3.735	-1.300
OLZ	4	3.93	96.19	4.465	-1.750
	8	7.88	96.49	4.098	-1.500
	10	9.85	96.41	4.349	-1.500
SLP	6	5.93	97.42	2.995	-1.166
	10	9.87	96.96	3.506	-1.300
	14	13.83	97.04	3.464	-1.214

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

Table 5: Application of the proposed method for the analysis of the studied drugs in spiked human urine

Drugs	Drug taken µg/ml	Drug found, µg/ml	Recovery[a], %	RSD, %	RE[b], %
ARZ	4	3.96	97.85	2.482	-1.000
	8	7.90	96.96	3.509	-1.250
	10	9.89	97.34	3.079	-1.100
CLZ	4	3.95	97.22	3.218	-1.250
	6	5.91	96.57	3.967	-1.500
	10	9.89	97.25	3.213	-1.100
OLZ	4	3.94	96.47	4.088	-1.500
	8	7.88	96.42	4.126	-1.500
	10	9.87	96.92	3.805	-1.300
SLP	12	11.9	98.50	1.745	-0.833
	16	15.8	98.21	2.072	-1.250
	20	19.8	97.90	2.418	-1.000

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

Effect of Interferences

In order to evaluate the selectivity of the proposed method for the analysis pharmaceutical formulations, the effects of the presence of excipients and additives, which can occur in real samples, were investigated. It was found that the presence of the common excipients of tablets such as talc, starch, gelatin, glucose, sulfate, acetate, phosphate and magnesium stearate did not interfere with

the determination of the studied drugs at the levels normally found in dosage forms.

Validation of the proposed method

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} and the concentration of the drug substances within the range 2.0 - 22 µg/ml. Regression analysis of Beer's law plotted at λ_{max} reveals a good correlation (r^2 = 0.9997 - 0.9998). 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 absorptivitys of the resulting colored complexes indicated high sensitivity of the method (4.57×10^4 - 10.3×10^4). The ARZ - MO 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.[67] The results are as shown in (Table 1).

Assay precision and accuracy

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 (Table 2) and indicate that the proposed method is highly accurate and reproducible.

Pharmaceutical applications

The proposed method was successfully applied to the analysis of ARZ, CLZ, OLZ and SLP in commercial tablets. The results of analysis of pharmaceutical formulations (Table 3) were compared statistically by Student t- test and by the variance ratio F- test with those obtained by reported methods. The Student t- values at 95% confidence level did not exceed the theoretical value indicating that there was no significant difference between the proposed and reported methods. It was also observed that the variance ratio F-values calculated for p=0.05 did not exceed the theoretical value indicating that there was no significant difference between the precision of the proposed and reported methods[21, 39, 46,54].

Biological applications

The high sensitivity of the proposed method, also allowed the in vitro determination of ARZ, CLZ, OLZ and SLP 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 in (Tables 4, 5) are satisfactorily accurate and precise.

CONCLUSION

The developed Spectrophotometric method was successfully applied to the determination of ARZ, CLZ, OLZ and SLP antipsychotics drugs in dosage forms and in biological fluids. The results obtained are compared statistically by student's t- test for accuracy and F- test for precision with the reported methods.[21,39, 46, 54] The results showed that the t- and F-values were less than the tabulated value indicating that there is no significant difference between the developed and the reported methods. Moreover, the developed method is more selective, sensitive, reproducible, rapid, cheap and simple. Therefore, the method developed here can be used for routine analysis in the majority of drug quality control laboratories where precision, time and cost effectiveness of analytical method is important.

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