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Determination of chlorzoxasone in presence of its alkaline degradation product with three spectrophotometric methods

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ABSTRACT

Three sensitive, selective and accurate methods are presented for the determination of chlorzoxazone (CHZ) in presence of its alkaline degradation product 2-amino-4-chlorophenol (ACP) and in its dosage form. Method A based on the ratio subtraction spectrophotometry by measuring the maximum absorbance of chlorzoxazone (CHZ) at 282.6nm after the subtraction of its degradant (ACP) interference. Beer's law was obeyed in the concentration range of 5.00-50.00μg.mL^{-1} with mean percentage recovery of 99.64 ± 1.036. Method B utilized the complexation reaction of the degradation product (ACP), after complete alkaline hydrolysis of chlorzoxazone (CHZ), with Cu (II) acetate solution to produce a colored complex measured after the second derivative at 477nm. Method C based on the oxidation-reduction reaction between the degradation product (ACP), after complete alkaline hydrolysis of chlorzoxazone (CHZ), and Folin-Ciocalteu reagent (F-C) to produce a blue color measured at 655.5nm. The linearity range was 2.50-20.00μg.mL^{-1} for both methods with mean percentage recovery of 99.81 ± 0.953 and 100.03 ± 0.947 for methods B and C, respectively. All variables were studied to optimize the reaction conditions. The proposed methods have been successfully applied to the analysis of chlorzoxazone in pharmaceutical dosage form. Results were statistically compared with the official method and no significant difference was observed regarding accuracy and precision.

KEYWORDS

Chlorzoxazone;
Spectrophotometry;
Stability-indicating methods;
Alkaline degradation;
Complexometry.

INTRODUCTION

Chlorzoxazone ($C_7H_4ClNO_2$), 5-chlorobenzoxazol-2(3H)-one, is a centrally acting skeletal muscle relaxant used for acute muscle spasm as well as for the treatment of chronic spasticity through the depression of reflex impulse conduction within the spinal cord[1-3], it is co-formulated with ibuprofen in the form of Myofen® capsules. The structures of chlorzoxazone, its alkaline degradant and ibuprofen are shown in figure 1.

The literatures reveal several methods for the determination of chlorzoxazone in presence of its degradation product and metabolites in biological fluids and drug combinations. These methods include HPTLC[4-8], GC[6,8-10], HPLC[11-24], RP-HPLC[25-27], CZE[28], titrimetry[29]. Oxidative coupling with MBTH, DMPD and DCQC was applied for stability indicating determination of chlorzoxazone in presence of its

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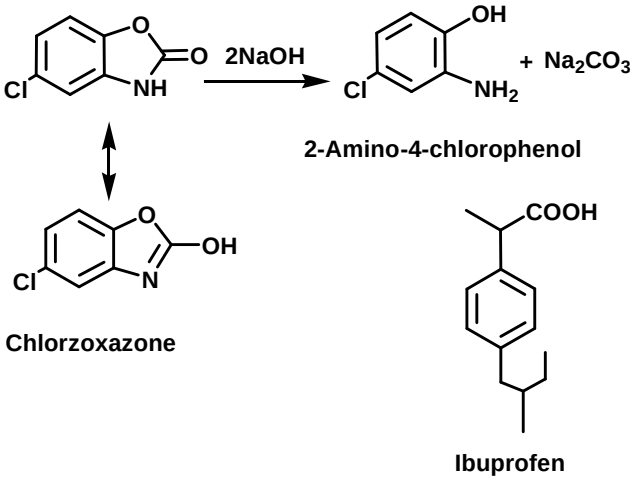

Figure 1 : Structures of chlorzoxazone, its alkaline degradant and ibuprofen

degradant[30]. Different spectrophotometric techniques used to determine chlorzoxazone in drug combinations, as simultaneous equations, first and second derivative, zero crossing derivative and the absorbance ratio technique[31-42].

The first method is the ratio subtraction which is very simple, accurate and precise and does not require any sophisticated apparatus or computer programs. Its main advantage is the direct measuring of the drug at its characteristic λ max, hence there is a potential for greater sensitivity and accuracy.

Theory of ratio subtraction method

The method depends on that, if you have a mixture of two drugs (X) and (Y) with overlapping spectra and the spectrum of (Y) is extended than (X), the determination of (X) can be done by dividing the spectrum of the mixture by a certain concentration of (Y) as a divisor (Y'). The division will give a new ratio spectrum that represents $\frac{X}{Y'}$ + constant. If we subtract this constant, then multiply the new spectrum obtained after subtraction by (Y') (the divisor), therefore we can obtain the original spectrum of (X). This can be summarized as follows:

$$\frac{X+Y}{Y'}=\frac{X}{Y'}+\frac{Y}{Y'}=\frac{X}{Y'}+\text{Constant}$$

$$\frac{X}{Y'}+\text{constant}-\text{constant}=\frac{X}{Y'}$$

$$\frac{X}{Y'}\ x\ Y' = X$$

The constant can be determined directly from the curve $\frac{X+Y}{Y'}$ by the straight line which is parallel to the wavelength axis in the region where (Y) is extended[43]. The correct choice of the divisor concentration is fundamental. If the concentration of the divisor is increased or decreased, the resulting constant value will be proportionally decreased or increased.

The complexometric method has the advantages of being sensitive and selective to the degradant of the drug. Thus, it can be used for the determination of the drug via degradation product in pharmaceutical laboratories for the routine analysis in both pure and dosage forms and for checking the extent of its degradation in pharmaceutical formulations. The colorimetric method depends upon the reaction between the degradant of chlorzoxazone and Folin-Ciocalteu reagent to produce a blue color at ambient temperature in the presence of sodium hydroxide solution that can be detected spectrophotometrically. The aim of the present work is to develop simple, selective and sensitive methods for the determination of chlorzoxazone in presence of its degradation product which is of special interest because it is one of the synthetic precursors of chlorzoxazone[44] and it was found to be toxic and classified as a harmful chemical since it interferes with the ability of blood to carry O_2 causing headache, methemoglobinemia and dizziness[45]. Ibuprofen is a co-formulated drug so; its interference was studied to simulate the pharmaceutical preparation.

EXPERIMENTAL

Apparatus

Spectrophotometer: Double beam UV-Visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cells of 1cm pathlength, connected to IBM compatible computer and HP 680 inkjet printer. The bundled software was UVPC personal spectroscopy software version 3.7. The spectra bandwidth was 2nm and wavelength scanning speed 2800nm/min.

Hanna pH meter: Model pH 211 with a combined glass saturated calomel electrode, Portugal.

Reagents

All chemicals and reagents are of pure analytical

grade.

(a) Methanol, acetonitrile and acetic acid of HPLC analytical grade (E.Merck, Germany).
(b) Acetate buffer pH range of 3.4-6, prepares it by mixing specific volumes of 0.01mol.L^{-1} acetic acid and 0.01mol.L^{-1} sodium acetate[46].
(c) Sodium hydroxide and hydrochloric acid, 2 mol.L^{-1}, aqueous solutions (Adwic) and distilled water.
(d) Copper (II) acetate (7.0% w/v, 4×10^{-4}mol.L^{-1}), dissolve the appropriate amounts of copper (II) acetate in recently boiled and cooled distilled water (E.Merck, Germany).
(e) Folin-Ciocalteu reagent, 2 mol.L^{-1}, (Fluka, Germany).

Samples

Pure sample: Chlorzoxazone and ibuprofen (IBU) pure substances were kindly supplied by EVA Pharma for Pharmaceuticals & Medical Appliances, Egypt. The purity of CHZ was assayed spectrophotometrically according to the USP 2005[47] official method and was found to be 99.88±0.651. The purity of IBU was assayed according to the BP 2004[48]. Official method "HPLC method using C_{18} column, phosphoric acid/acetonitrile/water (0.5: 340: 600 by volume) as a mobile phase and UV detection at 214 nm" and found to be 98.00±0.726.

Degraded sample: 2-Amino-4-chlorophenol was prepared by complete alkaline hydrolysis in sealed ampoules in oven at 120°C for 2 hours. It was also purchased from Sigma-Aldrich (Steinheim, Germany). The purity of 2-amino-4-chlorophenol was labelled to be 97%, it was also confirmed by evaluating its melting range and comparing it to that found in literature (139-143°C)[49].

Pharmaceutical dosage form: Myofen® capsules were purchased from Egyptian market. Each capsule is claimed to contain 250 mg of chlorzoxazone and 200mg of ibuprofen batch number 605453, 403144 and 303124. Myofen® capsules are manufactured by EVA Pharma for Pharmaceuticals & Medical Appliances, Egypt.

Standard solutions

(a) Chlorzoxazone in methanol (0.1mg.mL^{-1}).
(b) Degradant in methanol (0.1mg.mL^{-1}).
(c) Chlorzoxazone, degradant and ibuprofen in 2 mol.L^{-1} NaOH (0.625mg.mL^{-1}).
(d) Degradant in 2 mol.L^{-1} NaOH (4×10^{-4} mol.L^{-1}) for the determination of stoichiometry of the complex.
(e) Folin-Ciocalteu reagent 1:6 (F-C), dilute each 100mL of the reagent with 600mL of distilled water.

Procedures

Construction of calibration curves

Ratio subtraction method: Transfer accurately, aliquots containing 50-500µg from CHZ stock solution (0.1mg.mL^{-1}) into a series of 10-mL volumetric flasks then complete to volume with methanol. Transfer accurately aliquot equivalent to 450µg from the ACP stock solution (0.1mg.mL^{-1}) into 10-mL volumetric flask and complete to volume with methanol to be used as a divisor. Scan the spectra of the prepared standard solutions, construct a calibration curve relating the absorbance of the zero order spectra of CHZ at λ_{max} 282.6 nm to the corresponding concentrations in µg.$mL^{-1}$of CHZ and compute the regression equation.

Complexometric method: Transfer accurately, aliquots equivalent to 62.50-500.00µg of CHZ from stock solution (0.625mg.mL^{-1}) into a series of 2mL ampoules, then add 2 mol.L^{-1}NaOH to 1.5mL and seal the ampoules. Keep in oven at 120°C for 2 hours for complete degradation, then transfer the contents of the ampoules quantitatively into a series of 25-mL volumetric flasks, adjust the pH of the solutions to pH 7.0-7.5 by dropwise addition of 2 mol.L^{-1} HCl. Add 6mL 7.0% Cu (II) acetate solution, followed by 4mL acetate buffer pH 5.2±0.2. Leave for 45 min., then complete to volume with distilled water. Prepare blank for each concentration similarly but without the degradation step. Record the absorption spectra of the resulting solutions and the peak amplitudes of the second derivative spectra at 477nm of each concentration against its corresponding concentration prepared without hydrolysis (blank) using scaling factor = 1000 and $\Delta\lambda$ = 8. Plot the calibration curve between the peak amplitude at 477nm and its corresponding concentration and compute the regression equation.

Colorimetric method: Transfer accurately, aliquots equivalent to 62.50-500.00µg of CHZ from stock solution (0.625mg.mL^{-1}) into a series of 2mL ampoules, then add 2 mol.L^{-1}NaOH to 1.5mL and seal the am-

poules. Keep in oven at 120°C for 2 hours, then transfer the contents of the ampoules quantitatively into a series of 25mL volumetric flasks and add 3mL F-C reagent. Leave for 15 min. and then complete to volume with distilled water. Prepare blank for each concentration similarly but without the degradation step. Record the absorption spectra of the resulting blue colored solutions at λ_{max} 655.5 nm against the corresponding concentrations prepared without hydrolysis (blank). Plot the calibration curve between the absorbance at 655.5nm and its corresponding concentration and compute the regression equation.

Analysis of laboratory prepared mixtures

Ratio subtraction method: Into a series of 10mL volumetric flasks, transfer accurately complementary aliquots of CHZ from stock solution (0.1mg.mL^{-1}) and ACP stock solution (0.1mg.mL^{-1}), then complete to volume with methanol to prepare mixtures containing different ratios of CHZ and ACP. Scan the spectra of the prepared solutions and store in the computer. Divide the spectra of the laboratory prepared mixtures (absorbance at each wavelength) by the spectrum of 45μg.mL^{-1}of standard ACP (divisor) to obtain division spectra. Subtract the absorbance in the plateau region at λ above 302nm (the constant) from the division spectra, and then multiply the obtained curves (absorbance at each wavelength) by the spectrum of 45mg.mL^{-1} standard ACP. Use the obtained curve for direct determination of CHZ at 282.6 nm and calculate the concentration from the corresponding regression equation.

Complexometric method: Transfer accurate aliquots of CHZ, ACP and IBU stock solutions (0.625mg.mL^{-1}) with different ratios into a series of 2mL ampoules. Add 2 mol.L^{-1}NaOH to 1.5mL, seal the ampoules and follow the procedures detailed under construction of calibration curve. Prepare blank for each mixture similarly but without the degradation step. Record the absorption and the second derivative spectra for each laboratory mixture containing different percentages of CHZ, its degradation product (ACP) and IBU against its appropriate blank. Calculate the concentration of intact CHZ from the corresponding regression equation.

Colorimetric method: Transfer accurate aliquots of CHZ, ACP and IBU stock solutions (0.625mg.mL^{-1}) with different ratios into a series of 2mL ampoules. Add 2 mol.L^{-1}NaOH to 1.5mL, seal the ampoules and follow the procedures detailed under construction of calibration curve. Prepare blank for each mixture similarly but without the degradation step. Record the absorption spectrum of the blue colored solution for each laboratory mixture containing different percentages of CHZ and its degradation product against its appropriate blank. Calculate the concentration of intact CHZ from the corresponding regression equation.

Application to pharmaceutical preparation (Myofen® capsules)

Ratio subtraction method: Evacuate and weigh the contents of twenty capsules. Weigh an amount of the powder equivalent to 10mg CHZ into a beaker and add 50mL of methanol. Stir for 15 min. using a magnetic stirrer then filter into a 100mL volumetric flask. Wash the residue three times each with 10mL methanol and complete to volume with methanol. Transfer the calculated volume of the filtrate to a 1mL volumetric flask to make the appropriate dilution with methanol. Follow the procedures detailed under analysis of laboratory prepared mixtures (2.5.2.a).

Complexometric and colorimetric method: The same extraction procedure stated in ratio subtraction method using 2 mol.L^{-1} NaOH instead of methanol and then transfer the calculated volume of the filtrate that make the appropriate dilution into 2mL ampoules, add 2 mol.L^{-1}NaOH to 1.5mL, seal the ampoules and follow the procedures detailed under construction of calibration curve (2.5.1.b & 2.5.1.c) for complexometric and colorimetric analysis, respectively.

RESULTS AND DISCUSSION

Upon alkaline hydrolysis, CHZ produces ACP. It is a single component as indicated by the appearance of one spot after complete degradation and this was confirmed by TLC using ethyl acetate/benzene/acetic acid (20: 20: 1 by volume) as a mobile phase[4]. Its structure was also confirmed by IR spectrometry. Optimum conditions for the complete hydrolysis of CHZ were determined by studying many factors affecting the hydrolysis of CHZ as the effect of NaOH at room temperature, heat, sealed ampoules, normality and volume of NaOH. It was found that 2 mol.L^{-1}NaOH has no

hydrolytic effect on CHZ at room temperature but upon reflux, complete hydrolysis was achieved after five hours. The use of sealed ampoules reduces the time of complete hydrolysis from five hours to two hours. This is due to the high pressure produced in the ampoule by sealing and heating[50].

Ratio subtraction method

The absorption spectra of CHZ and ACP show some degree of overlapping, figure (2a), the theory of ratio subtraction was applied for complete separation by scanning the zero order spectra of the prepared standard solutions of CHZ in methanol, then the linearity was checked between the absorbance at the selected wavelength 282.6 nm and the corresponding concentrations of CHZ. Different divisor concentrations (15, 25, 35, 45 and 55μg.mL^{-1}) were tried. The divisor concentration 45μg.mL^{-1}was found the best regarding average recovery percent when the model was used for the calculation of CHZ concentration in its laboratory prepared mixtures.

Second, the scanning of the spectrum of the mixture (CHZ & ACP) in methanol, then divide it by a carefully chosen concentration of standard ACP (divisor) as shown in figure (2b). After the subtraction of the absorbance in the plateau at λ above 302nm as in figure (2c), multiply the obtained spectrum by the spectrum of the divisor (45μg.mL^{-1}) as shown in figure (2d).

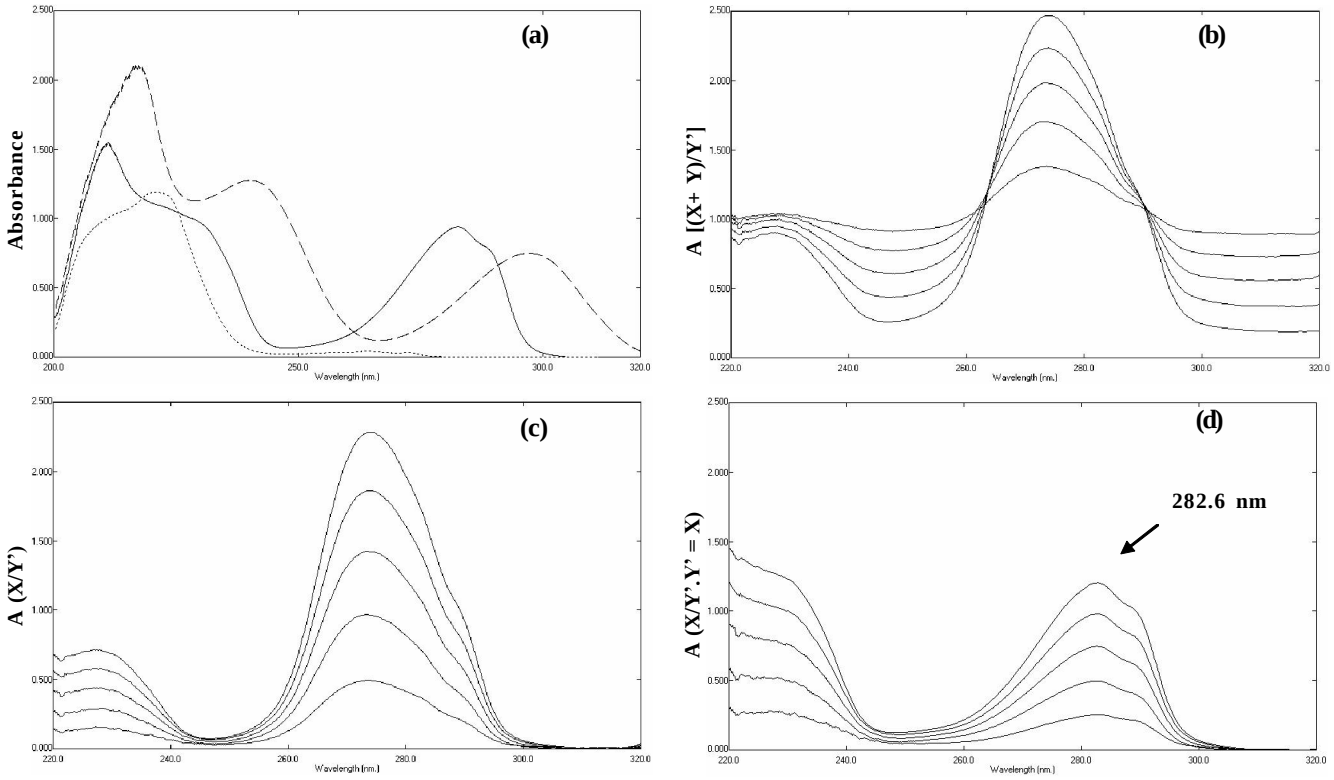

Figure 2 : (a) Zero order absorption spectra of chlorzoxazone ( ), 2-Amino-4-chlorophenol ( ) and Ibuprofen (…) using methanol as a blank. (b) Division spectra of laboratory prepared mixtures of chlorzoxazone (X) and 2-amino-4-chlorophenol (Y) using 45 μg/ml of 2-Amino-4-chlorophenol (Y') as a divisor and methanol as a blank. (c) Division spectra of laboratory prepared mixtures of chlorzoxazone (X) and 2-amino-4-chlorophenol (Y) using 45 μg/ml of 2-amino-4-chlorophenol (Y') as a divisor and methanol as a blank after subtraction of the constant. (d) The zero order absorption spectra of chlorzoxazone obtained by the proposed method for the analysis of laboratory prepared mixtures after multiplication by the divisor (Y')

Finally, use the last spectrum for direct determination of CHZ at 282.6 nm, and calculate the concentration from the corresponding regression equation. A linear relationship was obtained in the range cited in TABLE 1.

Complexometric method

The addition of aqueous Cu (II) acetate solution to ACP solution in pH 5.2±0.2 produces a green color insoluble in organic solvents and exhibits an absorption maxima at 438nm, on the other hand the aqueous Cu

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TABLE 1 : Analytical data for the determination of chlorzoxazone

Methods	Concn.range, μg.mL^{-1}	(Linear regression, A= bC+ a)*		
		Intercept (a)	Slope (b)	Correlation coefficient(r)
Ratio subtraction	5.00–50.00	0.0215	0.0298	0.9998
Complexometry	2.50–20.00	-0.0328	0.0744	0.9999
Colorimetry with F-C	2.50–20.00	-0.0389	0.0644	0.9999

***a is the analytical signal and C is the concentration in μg.mL^{-1}**

(II) acetate solution gives no color with CHZ but gives some degree of interference with IBU as shown in figure (3a). The second derivative spectrophotometry was used for clear separation of ACP at 477nm using scaling factor = 1000 and $\Delta\lambda$ = 8, this reaction gives the capability for the determination of CHZ via degradation product (ACP) by complexation with Cu (II) acetate using the second derivative spectrophotometry, figure (3b).

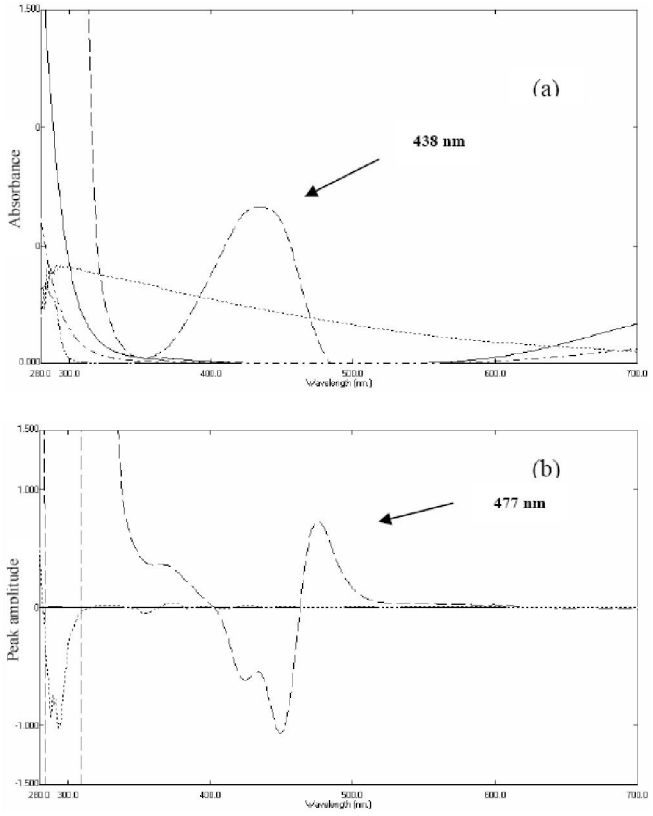
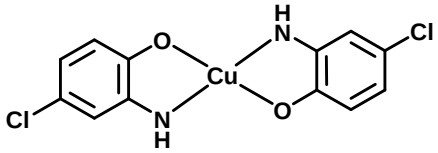


Figure 3 :(a) Absorption spectra of chlorzoxazone with copper (II) acetate solution (——), 2-amino-4-chlorophenol/ copper complex ( ), Ibuprofen/copper complex (…….), chlorzoxazone ( ) and copper (II) acetate solution ( ). (b) Second derivative spectra of chlorzoxazone with copper (II) acetate solution (——), 2-amino-4-chlorophenol/ copper complex ( ), Ibuprofen/copper complex (…….)

Various parameters affecting optimization of the reaction conditions were studied, as the reaction time, different pH range, the concentration of the reagent and the stability of the produced complex. It was found that the reaction required 45 min. in pH 5.2±0.2 with 6mL Cu (II) acetate aqueous solution (7.0% w/v) to reach its maximum color intensity which is stable over further one hour. A good linearity was obtained in the range cited in TABLE 1. The regression equation was calculated from the calibration graph. The application of the continuous variation method and the molar ratio method showed that ACP interacts with Cu (II) acetate in the ratio 2:1. Referring to Ringbom stability constant[51,52] $\beta \geq 10^2$, it is found that the constant of ACP/Cu (II) complex is well above this value; being 8.6×10^{10}. In the other words, all ACP was virtually chelated with Cu (II), and the complexation reaction is quantitative. The degradant was suggested to chelate Cu (II) yielding, five membered ring chelate as in figure 4.



2-Amino-4-chlorophenol / Cu (II) complex

Figure 4 : The suggested structure of the formed complex

Colorimetric method

It depends on measuring the absorbance of the blue color produced by the reaction between F-C reagent and ACP after complete alkaline hydrolysis of CHZ against the reagent with CHZ before hydrolysis as a blank at 655.5 nm without interference of neither CHZ nor IBU, since they can not react with F-C reagent that shows no absorbance at the wavelength of measurements as shown in figure 5. The formation of a blue colored chromogen is due to the reduction of phosphomolybdo tungestic mixed acid of the F-C reagent[53] by ACP, in the presence of sodium hydroxide, which can be measured at 655.5nm. The mixed acids in the F-C reagent have the following chemical species:

$3H_2O.P_2O_5.13WO_3.5MoO_3.10H_2O$

and $3H_2O.P_2O_5.14WO_3.4MoO_3.10H_2O$

ACP probably affects reduction of 1, 2 or 3 oxygen atoms from tungstate and/or molybdate in the F-C reagent, thereby producing one or more possible

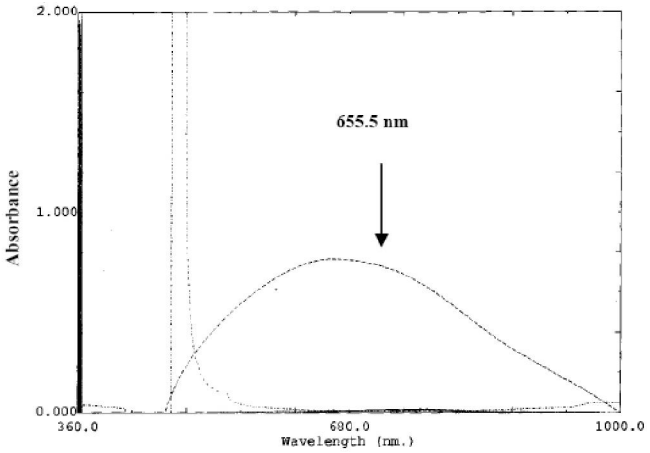


Figure 5 : Absorption spectra of 2-amino-4-chlorophenol with folin-ciocalteu reagent (…), chlorzoxazone with folin-ciocalteu reagent(….), Ibuprofen with folin-ciocalteu reagent (…) and folin-ciocalteu reagent (1:6) (…)

reduced species which have a characteristic intense blue color[54]. This mechanism was confirmed by the reaction of ACP with an oxidizing agent K_3 [Fe $(CN)_6$] which produced a quinone like structure and has no color and no absorbance in the region of measurement according to Woodward rule. The reaction of F-C reagent with a reducing agent ($SnCl_2$) gives also a blue color which indicates that the produced color is due to the transfer of electrons at basic pH to reduce the phosphomolybdic/phosphotungestic acid complexes. The effect of different variables was studied and optimized. It was found that the maximum color is developed within 15 min. of mixing the reactants using 1.5mL of 2 mol.L-1 NaOH and 3mL of F-C reagent (1:6), since at large volumes or concentrations of either NaOH or F-C reagent precipitation was observed. The produced color is stable for at least 50 min.

The validity and applicability of the proposed methods were assessed regarding accuracy, repeatability, intermediate precision and robustness (TABLE 2). The validity of the methods was indicated by the application of the suggested methods for the determination of chlorzoxazone in pharmaceutical formulation and the application of standard addition technique that revealed no interference from the excepients that may be found in the pharmaceutical preparation (TABLE 3).

Results of the determination of chlorzoxazone in laboratory prepared mixtures of the intact drug and its degradant in different ratios are presented in TABLE 4 showing high sensitivity and selectivity of the proposed methods as stability-indicating methods. TABLE 5 shows the statistical analysis of the results in comparison with the official method using student's t-test and F-ratio at 95% confidence limit and no significant difference was found.

TABLE 2 : Validation of the assay results using the proposed methods

Methods validation	Ratio subtraction	Complexometry	Colorimetry with F-C
Accuracy			
Range of linearity (μgml^{-1})	5.00–50.00	2.50–20.00	2.50–20.00
Mean±RSD%	99.64±1.040	99.81±0.955	100.03±0.947
Specificity & selectivity	99.81±1.110	99.53±0.481	99.17±0.527
Precision			
(Mean±RSD%)[a]	100.58±0.911	99.70±0.540	99.76±0.197
(Mean±RSD%)[b]	101.25±0.961	100.22±0.985	99.51±0.970
(Robustness)[c]	99.01±1.201	98.63±0.937	98.11±0.886
LOQ	1.356	0.538	0.621
LOD	0.448	0.177	0.205

[a]Intraday precision (average of 3 different concentrations (n=3) within the same day). [b]Interday precision (average of 3 different concentrations (n=3) repeated on 3 successive days). [c]A deliberate variation in method parameter were studied as variation of pH of buffer, volume of reagent used, time for complete reaction and concentration of divisor. LOD = (S.D of response/slope) 3.3. LOQ = (S.D of response/slope) 10

TABLE 3 : Determination of chlorzoxazone in pharmaceutical formulation by the proposed methods and application of standard addition technique

Preparation	Ratio substraction	Complexometry	Colorimetry with F-C
Myofen® capsules			
250 mg chlorzoxazone\capsule,Batch No. 605453	100.13±0.571	100.30±0.530	100.27±0.813
Standard addition			
Recovery±RSD%	100.17±0.858	100.22±0.775	99.60±0.265

TABLE 4 : Determination of chlorzoxazone in presence of its alkaline degradant in laboratory prepared mixtures by the proposed methods

Degradant%	Ratio subtraction	Complexometry	Colorimetry with F-C
25%	98.40	100.34	99.84
50%	99.75	99.33	98.80
75%	101.29	99.20	99.20
100%	100.41	99.60	98.40
125%	99.20	99.20	99.60
Mean±RSD%	**99.81±1.110**	**99.53±0.481**	**99.17±0.589**

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TABLE 5 : Statistical comparison between results obtained by applying the proposed and official methods for the determination of chlorzoxazone in pure powder form

Parameters	Ratio subtraction	Complexometry	Colorimetry with F-C	Official method[a]
Mean±SD	99.64±1.036	99.81±0.953	100.03±0.947	99.88±0.651
n	10	8	8	6
Variance	1.073	0.908	0.897	0.424
F	2.53(4.77)[b]	2.14(4.88)[b]	2.12(4.88)[b]	-
Student's t-test	0.506(2.145)[b]	0.154(2.179)[b]	0.397(2.179)[b]	-

[a]USP 2005 method "direct spectrophotometric determination of methanolic standard solution of CHZ at 282 nm and A(1%,1cm) = 305". [b]The figures in parenthesis are the corresponding tabulated values at P= 0.05

CONCLUSION

The proposed methods could be used as stability indicating methods, for the determination of drug via degradation product in pharmaceutical laboratories for routine analysis and quality control analysis of chlorzoxazone in both pure and dosage forms and for checking the extent of its degradation in pharmaceutical formulations due to their simplicity, accuracy and sensitivity.

The advantage of the proposed colorimetric methods over the other method is that these methods determine pure chlorzoxazone in presence of its degradant with high sensitivity and absorptivity.

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