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Spectrophotometric Determination of Allopurinol Drug in Tablets: Spectroscopic Characterization of the Solid CT Complexes

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Spectrophotometric micro determination of allopurinol (ALP) *via* charge-transfer formation is described. This includes the utility of some π-acceptors such as 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) and 3,6-dichloro-2,5-dihydroxy-*p*-benzoquinone (p-CLA) for estimation of ALP drug (act as è-donor). These reactions are applied for determination of ALP in its pharmaceutical preparations coming from different companies. Elucidation of the chemical structure of the solid CT complexes formed *via* reaction between drugs under study and π-acceptors, using elemental analyses (C, H, N), I. R., ^{1}H NMR and mass spectrometry.

Key Words: Allopurinol, DDQ, p-CLA, Spectrophotometry, Charge transfer complexes

Introduction

Allopurinol (ALP) and its mayor metabolite oxypurinol (OXP) are potent inhibitors of xanthine oxidase, the enzyme that converts hypoxanthine to xanthine, and xanthine to uric acid Figure 1. ALP is commonly used in the treatment of chronic gout or of hyperuricaemia associated with leukaemia, radiotherapy, anti-neoplastic agents and treatment with diuretics.[1] Procedures capable of simultaneously detecting the primary drug (ALP) and its metabolite (OXP) in body fluids are of considerable interest for pharmacokinetic and clinical studies. Several assays for the determination of both compounds have been reported using high-performance liquid chromatography (HPLC) with either ultraviolet[2-9] or electrochemical detection,[10,11] using ion exchange HPLC[2,3] and reversed phase HPLC.[4-11] Some of these methods have low limits of detection and quantitation, but others show various shortcomings, for example, the use of organic solvents (acetonitrile or methanol)[3] and excessively long elution times for ALP.[2] A capillary electrophoresis method for the determination of allopurinol and its active metabolite oxypurinol has been described.[12] Wang and co-workers[13] have reported a capillary zone electrophoresis (CZE) with end-column amperometric detection method using a running buffer composed of Na_2HPO_4/NaH_2PO_4 at pH 9.55 and detection potential at 1.20 V. Hempel and co-workers[14] have developed a CE assay with UV detection for the determination of ALP, OXP and other purine and products in urine with the aid of two running buffer formulations. In a first step, OXP was resolved by CZE using 60 mM sodium tetraborate at pH 8.7 as running buffer and, in a second step, micellar electrokinetic capillary electrophoresis (MEKC) with sodium dodecyl sulphate (80 mM) was used to resolve ALP.

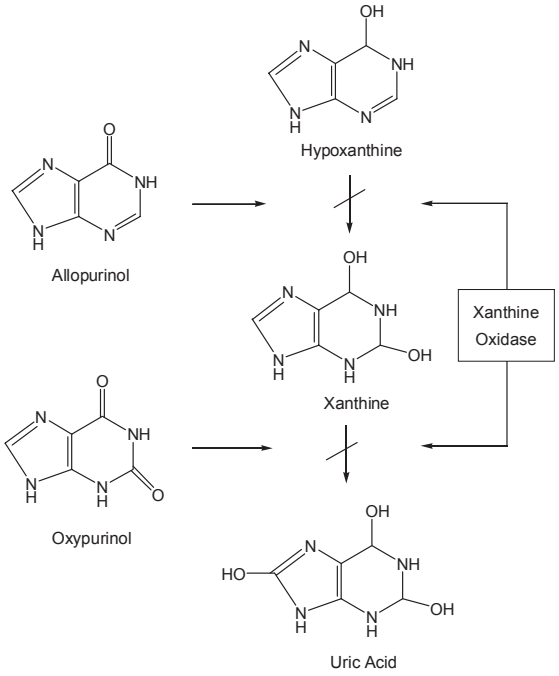


Figure 1. Diagram showing the inhibitory effect of allopurinol and oxypurinol for the formation of uric acid.

Accordingly, the aim of the present investigation was to optimize the spectrophotometric conditions for the determination of ALP in pure form and in pharmaceutical products. The effects of time, temperature, type of solvent, reagents concentrations and stoichiometry were carefully evaluated. The assay was validated by determining its accuracy, precision, linearity, specificity and sensitivity. The method has been successfully applied to the determination of ALP in pharmaceutical formulations. The isolated solid CT products were characterized using different physicochemical techniques.

Experimental

Materials. All chemicals and reagents were of analytical reagent grade and all of them were used as such without any further purification. They included allopurinol (ALP) that provided by EPICO. Reagents used included 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) supplied from Arcos-USA. While 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (p-CLA)

was supplied from BDH chemicals UK. Absolute ethanol and sodium hydroxide were supplied from ADWIC, while acetonitrile (AR) was supplied from Fisher chemicals and methanol was supplied from Sigma. Chloroform, acetone, 1,4-dioxane, methylene chloride, 1,2-dichloroethane and dimethyl formamide were supplied from El-Nasr Company.

The ALP pharmaceutical preparations were purchased from No-Uric capsules, 100 mg/cap. (EIPICO) and Zyloric, 100 mg/cap. (GlaxoSmithKline).

Solutions. 6.8×10^{-3} M ALP was prepared by dissolving the accurately weighed amount in alkaline methanol and completed to the mark with methanol. 0.1% (w/v) of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and 2,3-dichloro-5,6-dihydroxy-1,4-benzoquinone (p-CLA) reagents were prepared by dissolving 100 mg of each reagent in 100 mL acetonitrile. All solutions must be protected from light by keeping them in a dark colored quickfit bottles during the whole work. 0.1 M NaOH solution was prepared by dissolving 400 mg of NaOH in 100 mL methanol.

The water was always twice distilled from all glass equipments. Redistillation was carried out from alkaline permanganate solution.

Ten tablets of ALP were accurately weighed and the average weight of one tablet was calculated. The tablets were crushed well to a fine powder. A portion of the powder equivalent to 100 mg ALP was dissolved in 75 mL methanol, the solution was rendered alkaline by 0.1 M NaOH solution and then filtered on a dry filter paper in 100 mL volumetric flask. The volume was completed to the mark with methanol.

Equipments. All the absorption spectral measurements were made using the PerkinElmer automated spectrophotometer ranged from 200 - 900 nm with scanning speed 400 nm/min and band width 2.0 nm, equipped with 1cm matched quartz cells.

Elemental analyses (C, H, N) were determined at the Microanalytical center, Cairo University using CHNS-932 (LECO) Vario Elemental analyzers. Infra Red measurements (KBr discs) of the isolated CT complexes were carried out on PerkinElmer 1430 ratio recording Infrared Spectrometer (400 - 4000 cm^{-1}). ^1H NMR spectra in d_6-DMSO (200 MHz) were recorded on Varian spectrophotometers Gemini 200 using solvent signals as a reference. ^1H NMR data are expressed in parts per million (ppm). The mass spectra of the CT complexes were carried out at 70 eV by using EI-MS 30 mass spectrometer.

Procedures.

General procedure for spectrophotometric determination of ALP: 1 mL of 0.1% (w/v) DDQ and p-CLA was added to different concentrations of ALP (6.8×10^{-3} M). The mixtures were completed up to 10 mL with acetonitrile. The absorbance of the colored CT complexes was measured at the specific wavelengths against reagents blank prepared similarly without drugs.

Day-by-day precision: In order to prove the validity and the applicability of the proposed method and the reproducibility of the results obtained, four replicates experiments at different concentrations of ALP were carried out. Using the above mentioned procedures, the absorbance of the four samples were measured daily for four days and the results were recorded to make statistical calculations.

General procedure for spectrophotometric determination of ALP in some pharmaceutical preparations: Different concentrations of ALP drug (10 - 70 μg mL^{-1}) were added to 1 mL of 0.1% (w/v) DDQ or p-CLA. The volumes were made up to the mark with acetonitrile in 10 mL calibrated measuring flask. The absorbance was measured at λ_{max} = 450 and 515 nm for ALB-DDQ and ALP-p-CLA CT complexes, respectively, against reagents blank.

Synthesis of the charge transfer complexes: The solid CT complexes of ALP with DDQ and p-CLA were prepared by mixing saturated solution of the drug in chloroform (10 mL) and a saturated solution of DDQ or p-CLA in methanol (10 mL) with continuously stirring for about one hour at room temperature. The colored complexes developed and the solution was allowed to evaporate slowly at room temperature. Colored solid complexes were formed, filtered, washed several times with little amounts of methanol, and dried under vacuum over anhydrous calcium chloride.

Results and Discussion

Molecular charge-transfer complexes (CT) are of particular interest in pharmaceutical science. They can be applied as useful means in the qualitative and quantitative analysis of different pharmaceutical compounds.[15] A charge transfer complex is the name that given to a stable molecular system formed in solution between an electron donating molecule, having sufficiently low ionization potential, and an electron accepting molecule having high electron affinity.

The principal feature of this type of complex formation is the appearance of a new and intense absorption bands in ultraviolet or visible region of spectrum. Absorption bands of this type are known as charge transfer bands, since they involve electronic transitions from orbital on the donor to the vacant orbital on the acceptor. Many explanations were given to the phenomenon based on quantum mechanical theory of Mülliken. The formation of molecular complexes from two aromatic molecules could arise from the transfer of an electron from a π-molecular orbital of the donor (Lewis base) to a vacant π-molecular orbital of the acceptor (Lewis acid) i.e. π-π* electronic interaction.[16,17]

Absorption spectra. The absorption spectra of ALP-DDQ CT complex in acetonitrile solvent Figure 2 shows three maxima at λ = 450 nm ($\varepsilon_1 = 1.95 \times 10^3$ L·mol^{-1} cm^{-1}), 540 nm ($\varepsilon_2 = 0.80 \times 10^3$ L·mol^{-1} cm^{-1}) and 580 nm ($\varepsilon_3 = 0.69 \times 10^3$ L·mol^{-1} cm^{-1}). While the absorption spectra of ALP and DDQ shows no absorption peaks in the scanned region of spectrum. The peaks at λ = 450 nm was selected because it gives the highest absorption intensity as indicated from the ε value. The polar solvents such as acetonitrile and methanol were reported to promote complete transfer of electron from a donor (D) to the π-acceptor (A), [DDQ resulting in complete formation of DDQ radical anion (A^-) as a predominant chromogen.

$$\ddot{D} + A \longrightarrow \underset{\text{DA complex}}{[\ddot{D} - A]} \xrightarrow{\text{Polar solvent}} \underset{\text{Radical ions}}{D^+ + A^-}$$

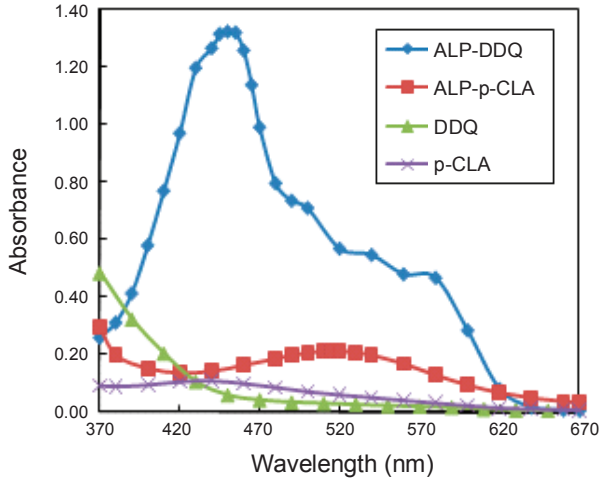


Figure 2. Absorption spectra of DDQ and p-CLA in methanol and their charge transfer complex with ALP drug.

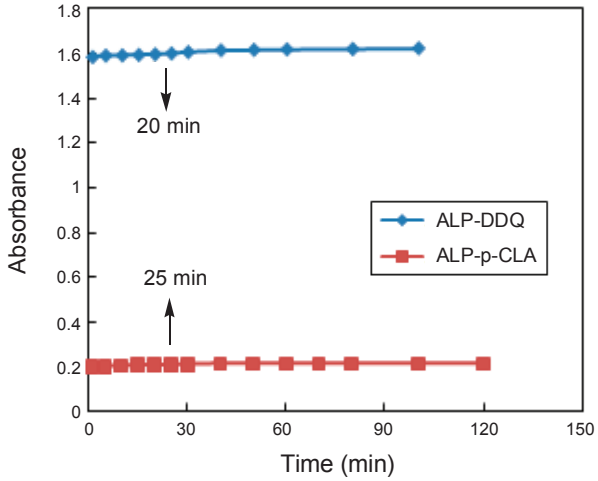


Figure 3. Effect of time on the absorbance of CT complex of ALP drug with DDQ and p-CLA in acetonitrile.

Table 1. The molar absorptivity values of ALP drug with DDQ or p-CLA reagents in different solvents

Solvent	Absorbance		ε (L·mol^{-1} cm^{-1}) × 10^3	
	λ = 450 nm	λ = 515 nm		
	DDQ	p-CLA	DDQ	p-CLA
Acetonitrile	0.838	0.000	1.23	0.00
Methanol	0.779	0.103	1.15	0.08
Ethanol	0.963	0.108	1.42	0.08
Acetone	0.855	0.073	1.26	0.05
Dichloromethane	0.000	0.000	0.00	0.00
1,2-Dichloroethane	0.000	0.000	0.00	0.00
DMF	0.741	0.117	1.09	0.09
Chloroform	0.000	0.000	0.00	0.00
1,4-Dioxane	1.126	0.000	1.66	0.00

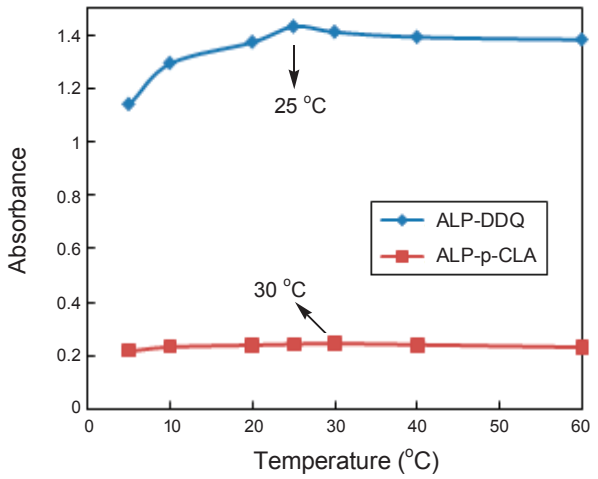


Figure 4. Effect of temperature (5 - 60 °C) on the absorbance of CT complex of ALP drug with DDQ and p-CLA in acetonitrile.

Figure 2 shows the absorption spectra of ALP-p-CLA in acetonitrile (an intense pink color) which has a characteristic wavelength absorption band, frequently with one maxima at λ = 515 (ε = 0.16 × 10^3 L·mol^{-1} cm^{-1}), while p-CLA solution showed a peak at λ = 440 nm (ε = 0.23 × 10^3 L·mol^{-1} cm^{-1}).

Effect of solvents. In order to select the suitable solvent for CT complex formation, the reaction of DDQ and p-CLA with ALP drug is made in different solvents. These solvents include acetonitrile, chloroform, ethanol, methanol, acetone, 1,4-dioxane, dichloromethane, 1,2-dichloroethane and dimethyl formamide (Table 1). For ALP drug, 1,4-dioxane, ethanol and acetone have high molar absorptivity than acetonitrile in case of DDQ reagent, while in case of p-CLA, dimethyl formamide, ethanol and methanol have high molar absorptivity while acetonitrile shows turbidity.

Effect of reagents concentrations. It is found that, when various concentrations of DDQ or p-CLA solutions are added to a constant concentration of ALP drug, it is obvious that 1000 μg mL^{-1} of DDQ or p-CLA solutions, respectively, is found to be sufficient for quantitative determination of the drug under study as mentioned, respectively. It also means that, maximum and reproducible colour intensities are obtained and higher concentration of reagents does not affect the colour intensity.

Effect of time and temperature. The optimum reaction time and temperature are determined spectrophotometrically at different time intervals and at λ_{max} = 450 and 515 nm for ALP-DDQ and ALP-p-CLA CT complexes, respectively. Figures 3 and 4, shows that complete color development is attained after (20 min and 25 ± 1 °C) and (25 min and 30 ± 2 °C) for ALP-DDQ and ALP-p-CLA CT complexes, respectively.

Stoichiometry of the CT complexes.[18,19] Molar ratio and Job's continuous variation methods are applied in order to determine the suitable ratio between ALP drug with DDQ or p-CLA reagents. Figures 5 and 6 shows that the interaction between this drug and reagents occurs in equimolar basis, i.e. the two straight lines are intersected at 1:1 [Drug]:[Reagents]. This means that, 1:1 complexes were formed between the drug and DDQ or p-CLA reagents. The CT complexes formed between DDQ or p-CLA and ALP drug takes place through the transfer of electron from a donor (drug) to the π-acceptor reagent (DDQ

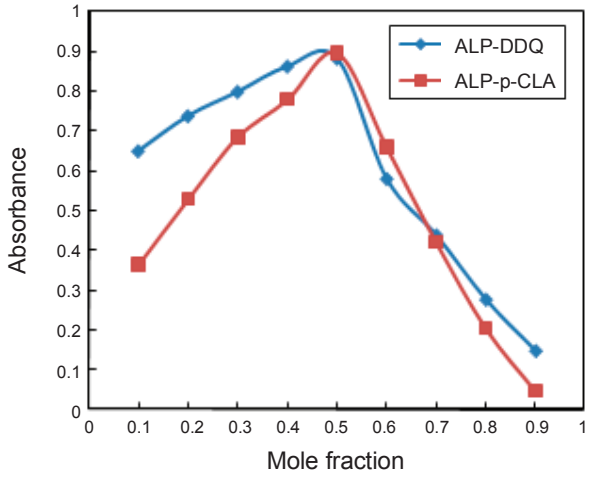


Figure 5. Job's method for ALP CT complexes with DDQ and p-CLA in acetonitrile.

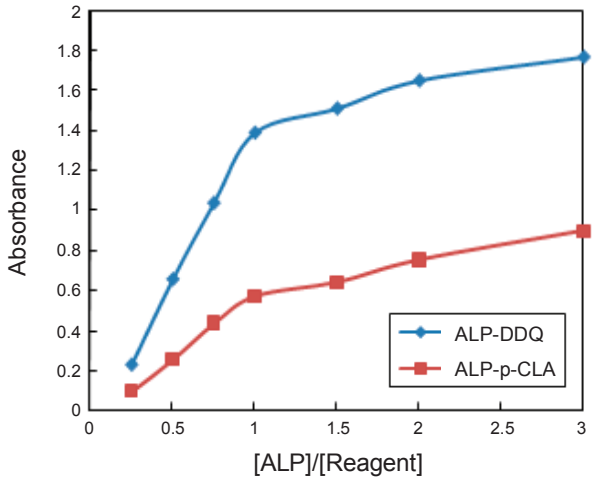


Figure 6. Molar ratio of ALP CT complexes with DDQ and p-CLA in acetonitrile.

Table 2. Spectral characteristics of allopurinol CT colored reaction products and the analytical characteristics (accuracy and precision) of these reactions

Parameters	Results	
	DDQ method	p-CLA method
λ_{max}, nm	450	515
Molar absorptivity, L·mol^{-1}. cm^{-1}	1.95×10^3	0.16×10^3
Sandell Sensitivity, µg cm^{-2}	0.0035	0.0043
Beer's law limit, µg mL^{-1}	2.50 - 60.00	5.00 - 50.00
Percentage recovery, %	98.40 - 100.7	98.20 - 100.4
Range of error, %	0.03 - 1.6	0.04 - 1.80
Standard Deviation (SD)	0.07 - 0.48	0.18 - 0.67
Relative Standard Deviation, (RSD) %	0.12 - 0.74	0.18 - 0.94
Regression equation,[a] slope (b)	0.0189	0.0150
Intercept (a)	0.2460	−0.0142
Correlation coefficient (r^2)	0.9866	0.9988
LOD, µg mL^{-1}	7.96	1.70
LOQ, µg mL^{-1}	26.53	5.68

[a]A = a + bC; where C is the concentration in µg mL^{-1}

Table 3. Between − day precision of the determination of ALP drug using DDQ or p-CLA reagents under optimum conditions

Reagent	[Drug] Taken, µg mL^{-1}	[Drug][a] Found, µg mL^{-1}	Percentage Recovery (%)	SD	RSD (%)
DDQ	5.00	5.00	100.0	0.07	1.36
	20.00	20.03	100.1	0.14	0.70
	30.00	30.13	100.4	0.31	1.04
	60.00	59.99	99.98	0.22	0.37
p-CLA	10.00	9.82	98.20	0.18	1.49
	15.00	15.20	101.4	0.32	1.51
	20.00	20.19	101.0	0.40	1.77
	25.00	24.76	99.00	0.25	0.92

[a]The average of four replicates.

or p-CLA).[18]

Validity of Beer's law. After the selection of suitable solvents, reagent concentrations, reaction time, temperature, and ratio of reactants it is also important to know the concentration limits of ALP drug at which these reactions are quantitative. It is found that, Beer's law is valid over the concentration ranges from 2.5 - 60 and 5 - 50 µg mL^{-1} of ALP using DDQ and p-CLA reagents, respectively. Table 2 shows the slope, intercept, correlation coefficient, Sandell sensitivities, molar absorptivity (ε), range of error, standard deviation, relative standard deviation, limits of detection (LOD) and quantification (LOQ). The small values of Sandell sensitivity indicate the high sensitivity of the proposed method in the determination of the drug under investigation.

Four to six replicates measurements are performed at different concentrations of ALB, ALP and SILC drugs using DDQ and p-CLA reagents. The relative standard deviation and the range of error values are calculated and found that the small values of them indicate the high accuracy and high precision of the proposed spectrophotometric method. The low values of limits of detection (LOD) and quantification (LOQ) indicate the possibility of applying DDQ and p-CLA reagents in routine analysis of the drugs under investigation.

Between-day precision. In order to prove the validity and applicability of the proposed method and reproducibility of the results obtained four replicates experiments at four concentrations of ALP are carried out. Table 3 shows the values of the between-day relative standard deviations for different concentration of the drugs, obtained from experiments carried out over a period of four days. It is found that, the within day relative standard deviations are less than 1%, which indicates that the proposed method is highly reproducible and (DDQ and p-CLA) reagents are successfully applied to determine ALP *via* the charge transfer reaction.

Spectrophotometric micro determination of ALP drug in different pharmaceutical preparations. The spectrophotometric micro determination of ALP drug *via* its reaction with DDQ and p-CLA reagents, coming from EPICO and GlaxoSmith-Kline Companies are carried out. The results obtained are given in Table 4. These data show that, the determined concentration

Table 4. Spectrophotometric determination of ALP drug in different pharmaceutical preparations using DDQ or p-CLA and official methods

Samples	[Taken] μg mL^{-1}	[Drug] μg mL^{-1}		Percentage recovery (%)		SD[a]	SD[b]	F-test	t-test
		DDQ method	Official method	CLA method	Official method				
	15.00	15.17	15.99	101.1	106.6	0.08	0.13	0.38	0.21
Using DDQ: ALP	30.00	29.86	29.91	99.53	99.70	0.10	0.21	0.23	1.00
	45.00	45.18	44.95	100.4	99.89	0.12	0.13	0.85	0.38
	15.00	14.54	15.45	96.93	103.0	0.08	0.78	0.01	2.27
D1	30.00	30.87	29.95	102.9	99.83	0.15	0.07	4.59	1.22
	45.00	44.87	45.04	99.71	100.1	0.24	0.19	1.60	1.41
	15.00	15.09	15.32	100.6	102.1	0.18	0.37	0.24	2.56
D2	30.00	29.98	30.19	99.93	100.6	0.22	0.46	0.23	1.91
	45.00	45.14	45.33	100.3	100.7	0.21	0.37	0.32	1.81
	15.00	15.21	15.99	101.4	106.6	0.32	0.13	6.06	0.49
Using p-CLA: ALP	30.00	29.76	29.91	99.20	99.70	0.25	0.21	1.42	1.20
	45.00	45.03	44.95	100.1	99.89	0.48	0.13	1.36	0.33
	15.00	14.78	15.45	98.53	103.0	0.73	0.78	0.88	1.84
D1	30.00	29.78	29.95	99.27	99.83	0.95	0.07	1.84	0.36
	45.00	45.22	45.04	100.5	100.1	0.40	0.19	4.43	0.90
	15.00	15.07	15.32	100.5	102.1	0.56	0.37	2.29	0.89
D2	30.00	30.26	30.19	100.9	100.6	0.89	0.46	3.74	0.16
	45.00	45.11	45.33	100.2	100.7	0.44	0.37	1.41	1.00

No. of replicates (n) = 4.
D1 No-Uric tablets (100 mg/cap.), EPICO, Cairo, Egypt.
D2 Zyloric tablets (100 mg/cap.), GlaxoSmithKline, Cairo, Egypt.
Standard F-values at 95 % confidence level = 6.39. Standard t-values at 95% confidence level = 2.77
[a]Standard deviation values using proposed method. [b]Standard deviation values using official method.

Table 5. Elemental analysis (C, H, N) and physical parameters data of the CT-complexes formed from the reaction of the ALP drug with DDQ or p-CLA reagents

Complexes (FW)	M wt (g/mol)	C%	H%	N%	Physical data	
	Found	Found	Found	Found	Color	mp (°C)
	Calculated	Calculated	Calculated	Calculated		
ALP-DDQ	328.0	42.77	1.07	23.08	Yellow	215
($C_{13}H_4N_6Cl_2O_3$)	363.1	42.96	1.10	23.13		
ALP-p-CLA	302.0	38.11	2.54	16.15	Dark red	218
($C_{11}H_9N_4Cl_2O_5$)	344.1	38.36	2.62	16.27		

of ALP drug by the proposed methods are close to that obtained from the applied standard method.[20,21]

In order to check the confidence and correlation between the suggested spectrophotometric procedures and the official method[20,21] for micro determination of ALP drug, it is better to do the F- and t-tests (Table 4). The calculated F- and t-tests at the 95% confidence level do not exceed the theoretical values indicating that there is no significant difference between the proposed and official methods. The small values of SD and RSD indicate the reliability, accuracy and precision of the suggested procedures.

Table 4 shows the results obtained by determining the different concentrations of ALP drug using DDQ and p-CLA reagents. It is obvious from these results that, the percent recoveries are found to be 99.53 - 101.1 and 99.20 - 101.4% with DDQ and p-CLA, respectively. These values indicate the accuracy and precision using DDQ and p-CLA reagents.

Characterization of charge-transfer (CT) complexes. Charge-transfer (CT) complexes formed between ALP drug as donor with DDQ and p-CLA as acceptors have been isolated in solid form. The synthesis and characterization of ALP-DDQ and ALP-p-CLA were described. These complexes are readily prepared from the reaction of ALP with DDQ and p-CLA within chloroform and/or methanol solvents. IR, ^{1}H NMR, mass spectra and elemental analyses (C, H, N) were performed to characterize the charge-transfer complexes.

Compositions and solubility of the CT-complexes: Results of elemental analysis for all the ALB, ALP and SILC CT complexes are listed in Table 5. From the table, it can be seen that values found are in a good agreement with the calculated ones, and the composition of the CT-complexes is matched with the stoichiometry (1:1; drug: reagents) which is examined by applying continuous variation and molar ratio presented from a series of solutions of DDQ or p-CLA to ALP. All the CT-complexes

Table 6. Infrared frequencies[a] (cm^{-1}) and tentative assignments for DDQ, p-CLA, ALP-DDQ and ALP-p-CLA CT-complexes

DDQ	p-CLA	ALP	Assignments[b]		
			ALP-DDQ	ALP-p-CLA	
3325 w 3218 br	3237 s, br	3166 w 3082 w 3042 w	3422 m 3161 w, sh 3084 m	3423 w, br 3353 m 3165 vw 3081 vw 3041 vw	$\nu_{(O-H)}$ $\nu_{(N-H)}$ NH_2^+
-	-	2991 w 2944 w 2873 m	2863 s, br	2991 vw 2942 vw	$\nu_{s(C-H)} + \nu_{as(C-H)}$
-	-	-	2800-2600 w, sh	2865 s, br 2691 w	Hydrogen bonding
2250 vw 2231 ms	-	-	2227 ms	-	$\nu_{(C\equiv N)}$
1673 vs	1664 vs	1699 s	1704 vs	1687 vs	$\nu_{(C=O)}$
1552 vs 1451 s	1630 vs	1588 s 1478 s	1587 vs 1478 sh 1450 s 1391 w	1576 vs 1479 ms 1386 w	δ_{def}(N-H); pyrazole ν(pyrazole ring) Ring breathing bands $\nu_{(C=C)} + \nu_{(C=N)}$ + COO-C-H deformation+NH_2^+ Ring breathing bands
1358 w 1267 s 1172 vs 1072 w	1366 s 1267 s, br	1387 s 1363 s	1359 s 1233 s 1188 s 1077 s	1361 s 1232 vs 1108 vs	$\nu_{(C-C)} + \nu_{(C-N)} + \nu_{(C-O)}$ ν(pyrimidine ring)
1010 vw 893 s	980 vs 847 vs	1231 vs 1156 ms 1080 vs	951 s 887 vs	952 ms 908s	δ_{rock}; NH $\nu_{(C-N)}$; pyrazole $\nu_{(C-C)}$ CH, in-plane bend CH-deformation $\nu_{(C-Cl)}$
800 vs 720 s 615 ms 527 vw 457 ms 432 mw	753 s 688 s 566 s	954 vs 912 w 885 w 813 ms 779 ms 703 vs 602 vs 535 vs	816 s 776 s 701 s 600 vs 533 s	813 mw 777 w 749 s 705 s 600 s 534 ms 471 vw	skeletal vibration CH bend CH out-of-plane bend Skeletal vibration NH-out of plane CNC def. NH_2 rock

[a]s = strong, w = weak, m = medium, sh = shoulder, v = very, br = broad. [b]ν, stretching; δ, bending.

are insoluble in cold and hot water, but easily soluble in dimethyl formamide and dimethyl sulfoxide.

IR spectral studies:

In case of ALP-DDQ CT-complex − The IR spectra of the molecular complex of DDQ with ALP Table 6 indicate that the ν(C≡N) and ν(C-Cl) of the free acceptor are shifted to lower wavenumber values on complexation. IR spectrum of the molecular complex of DDQ with ALP indicate that the band of ν(C≡N) of the free acceptor molecule which exhibited at 2250 cm^{-1} is shifted to a lower wavenumber value 2227 cm^{-1}, while the ν(C=O) absorption band of the free DDQ at 1673 cm^{-1} is shifted to higher value 1687 cm^{-1}. IR spectra strongly confirmed that the CT-interaction in case of ALP-DDQ complex occurs through n-π* transition with deprotonation of −NH group of ALP to only one of the CN groups by forming intermolecular hydrogen bonding. The characteristic bands of the hydrogen bonding are appearing in the IR spectrum of the resulted complex and ranged from 2600 to 2800 cm^{-1}. These bands are not existed in both spectra of the free donor and the DDQ acceptor. The intensities of δ_{def}(N-H); pyrazole and ν(pyrazole ring) are hyperchromically affected, this mean that pyrazole ring sharing in the CT complexation with DDQ *via* NH group (Scheme 1).

In the case of ALP-p-CLA CT-complex − The IR spectra of the ALP/P-CLA CT complex is characterized by a detected bands appearing at 2865 and 2691 cm^{-1}, which are not present in the spectra of the free donor and acceptor. These bands are attributed to the stretching vibration of a proton attached to the donation site of the donor.[22] These results can be attributed to the protonation of the $^+$NH group of the donor through one protons transfer from the two of the acidic center on the p-CLA acceptor from one (OH) sides to the basic center on the donor $^+$NH group. Such assumption is strongly supported by the appearance of an absorbance band at 1576 cm^{-1} due to $^+NH_2$ deformation, and the bands near 800 cm^{-1} which attributed to

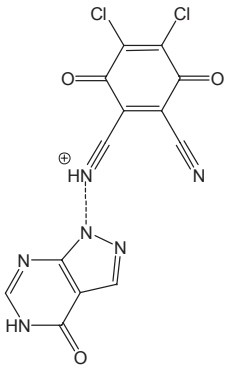
Table 7. ^{1}H NMR spectral data of ALP, DDQ, p-CLA, ALP-DDQ and ALP-p-CLA CT complexes

Compound	Chemical shift δ (ppm)	Assignments
ALP	3.846 (s) 7.976 + 8.132 (s) 12.872 (br)	1H; −NH (pyrimidine ring) 2H; aromatic ring (Pyrimidine and pyrazole rings) 1H; −NH (pyrazole ring)
DDQ	-	-
p-CLA	8.90 (br)	2H; 2(OH)
ALP-DDQ	4.015 (s) 8.010 + 8.138 (s) 12.042 (s)	1H; −NH (pyrimidine ring) 2H; aromatic ring (Pyrimidine and pyrazole rings) 1H; −NH (pyrazole ring)
ALP-p-CLA	3.744 (s) 7.984 + 8.130 (s) 11.993 (s)	1H; −NH (pyrimidine ring) 2H; aromatic ring (Pyrimidine and pyrazole rings) 1H; −NH (pyrazole ring)

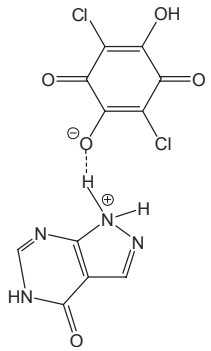
Table 8. Mass fragmentation of ALP, ALP-DDQ and ALP-p-CLA CT complexes

Compound	*m*/*z* (%)[a]
ALP	136 (96%), 67 (17%), 52 (100%)
DDQ	227 (53%), 200 (64%), 165 (15%), 137 (37%), 110 (45%), 87 (100%), 52 (55%)
p-CLA	209 (49%), 188 (49%), 145 (23%), 123 (12%), 105 (43%), 87 (56%), 69 (100%), 52 (43%)
ALP-DDQ	328 (9%), 200 (16%), 136 (100%), 97 (16%), 52 (75%)
ALP-p-CLA	302 (1%), 136 (100%), 52 (82%)

[a]Intensities expressed as % of base peak.



Scheme 1. Structure of the ALP-DDQ CT-complex



Scheme 2. Structure of the ALP-p-CLA CT-complex

NH_2 rock. This is further supported by disappear or decrease in the stretching of OH group of p-CLA due to intermolecular hydrogen bond forming. Accordingly, the hydrogen bonding interaction between the donor and the acceptor can be formulated as Scheme 2. The bands of the donor and acceptors in the mentioned complexes reveal small shifts in both band intensities and wavenumber values from those of the free reactants. These phenomena back to the change in the electronic configuration upon the CT complexation.

^{1}H NMR spectral studies: ^{1}H NMR spectra of ALP drug and its CT-complexes in DMSO are measured and the assignments of spectral data are listed in Table 7. Evidently, the results obtained from elemental analyses, infrared spectra, and molar ratio titrations met in the same point with ^{1}H NMR spectra to interpret the mode of interaction between donor and acceptor. It is clear that the signal of H of −NH of pyrazole ring (12.87 ppm) which exists in the free donor ALP is shifted to 12.04 for ALP-DDQ and 11.99 for ALP-p-CLA, respectively. This up field shift confirm the place of interaction between donor and acceptor *via* intermolecular hydrogen bond between −NH of pyrazole ring with CN and OH groups for DDQ and p-CLA, respectively.

Mass spectra: Mass spectrometry has been applied in order to study the purity and the main fragmentation routes of ALP charge-transfer complexes. The differentiation in fragmentation were caused by the nature of the attached acceptors through the intermolecular hydrogen bond between donor/acceptor, while the molecular ion peaks characterized to DDQ *m*/*z* = (M+1) 228 (53%), p-CLA *m*/*z* = 208 (49%), ALP *m*/*z* = 136 (96%) are detected in the fragmentation of their CT-complexes. The corresponding mass spectra are given in Table 8. The different competitive fragmentation pathways of donors give the peaks at different mass numbers listed in Table 8. The intensities of these peaks reflects the stability and abundance of the ions.

Conclusion

Simple, rapid and reliable spectrophotometric methods were adopted for the micro determination of ALP drug *via* CT complex formation with DDQ or p-CLA reagents spectrophotometrically. The effect of different parameters was studied. The results obtained by the suggested procedure were compared with those obtained by the standard method. The data obtained by both procedures were found to be very close to each other and very close to those given by the pharmaceutical companies. The calculated F- and t-tests at the 95% confidence level do not exceed the theoretical values. The results obtained by the suggested procedure were compared with those obtained by the standard method. The data obtained by both procedures were found to be very close to each other and very close to those given by the pharmaceutical companies. The calculated F- and t-tests at the 95% confidence level do not exceed the theoretical values.

Also, the formed CT complexes were studied using elemental analyses, IR, ^{1}H NMR and mass spectrometry in order to elucidate the structure of these CT complexes. The results obtained confirm the results of stoichiometry studied before and suggested that 1:1 reaction between donors and acceptors under study, in addition it helped in elucidation of the site of interaction between donors and acceptor.

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