Egypt. J. Anal Chem., Vol. 15, pp.82-89 (2006)

Quantitative Determination of Dopamine Hydrochloride and Levodopa in Pharmaceutical Forms Using Simplified Spectrophotometric Method

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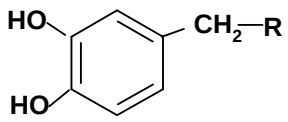
Summary. Simple, accurate and sensitive spectrophotometric method was reported for determining dopamine hydrochloride (DO.HCl) and levodopa (LD) in either pure forms or in their commercially available pharmaceutical formulations. The main idea of the method based on reacting a solution of DO.HCl or LD at pH 12; using universal buffer, with 4-aminoantipyrine (4-AAP) to form pink coloured coupling dye product with λ_{max} = 475 or 454 nm, for DO.HCl and LD drugs, respectively. Before carrying out Beer's law, different experimental conditions like time, temperature, sequence of addition and pH were optimized. The molar ratio method revealed a 1:1 [active ingredient]:[4-AAP] coupling product. The common excipients used as additives in pharmaceuticals were examined in our proposed procedure as interfering materials. The calibration curves were developed by using standard DO.HCl and LD with percent recovery of 98.6–102 %. The Beer's law was found to be in the range from 37.9–170.6 and 49.3–221.8 mg\L of DO.HCl and LD, respectively. The sandell sensitivity and molar absorptitivity values were found to be $3.9x10^{-3}$, $2.9x10^{-3}$ μg cm^{-2} and $3.375x10^{4}$, $2.43x10^{4}$, for DO.HCl and LD, respectively. The reproducibility and accurate of the method was checked by the values of SD (0.27 and 0.26) and RSD (0.76% and 0.71%) for DOHCl or LD, respectively. The results obtained were compared favorably with those of official and reported methods as indicated by the t- and F-test values.

Introduction

Dopamine and dopamine derivatives were a group of biogenic amines possessing a 3,4-dihydroxy substituted phenyl ring (Figure 1). They considered as a type of hormones widely spread in animals and had also been detected in 44 plant families.[1,2]



DO; R: CH_2 CH_2 (NH_2)

LD; R: $CH(NH_2)COOH$

Fig. 1. The structural formula of dopamines

They also seemed to be a central pharmacophore and well probably existed in future drugs, especially in those developed for psychiatry disorders and neurological activity.[3] It was not until the late 1950's that dopamine was recognized as a mamalian neurotransmitter in its own right but the demonstration of its non-uniform distribution in the brain suggested that it might has a specific functional role for dopamine.[4] It had therapeutic uses as a cardiostimulant and had important role in the pathogenesis or drug treatment of certain brain diseases e.g. Parkinson's disease and Schizophrenia.[5]

Several methods were applied on pharmaceutical preparations containing DO.HCl or LD depending on oxidation reaction.[6-8] Determination of certain catechol derivatives likes pyrocatechol, DO.HCl and LD in either pure form or in its pharmaceutical formulation was suggested spectrophotometrically[9-11] and indirect kinetic spectrophotometry.[12]

HPLC technique most predominantly used for screening of many clinical diagnosis[13 -15] and to find difference between the measured and ordered dose of catecholamine infusion.[16] Flow injection analysis (FIA) system using tubular electrode was used in the determination of DO in pharmaceutical preparations. The process based on redox properties of copper (II) ions immobilized in a poly (ethylene-co-vinyl acetate) (EVA) membrane and oxidation of DO.[17]

In continuation to our interest in microdetermination of these drugs under study[18]**,** the aim of the present work is to describe the development of simple, sensitive and rapid spectrophotometric method for the determination of DO.HCl and LD depending upon the formation of coloured chelate products with 4-AAP. Different experimental conditions are carefully studied before applying Beer's law. The method was applied for determining DO.HCl and LD in pure and pharmaceutical forms and the results obtained are of interest and compared with these obtained by the official method.

Experimental

An aliquot containing 37.9-170.7 or 49.3-221.8 ppm of DO.HCl or LD, respectively was transferred to 10 mL measuring flask, followed by adding 10^{-2} M 4-

AAP. The pH was adjusted using universal buffer of pH = 12. The total volume was completed up to 10 mL. The mixtures were shacked well and allowed to stand at 30 ± 2 and 38 ± 2 ^{0}C for 30 and 38 minutes, The pH was rechecked and the absorbance was measured at 475 and 454 nm for DO.HCl and LD, respectively, against de-ionized water as a blank. The calibration curves were obtained applying the same procedure using standard solutions of active ingredient.

Results and Discussion

4-AAP reacts with phenolic type compounds according to the reaction show in Fig.2. The reaction product may be any colour from red to purple depends on the phenolic type compounds involved.[20-22]

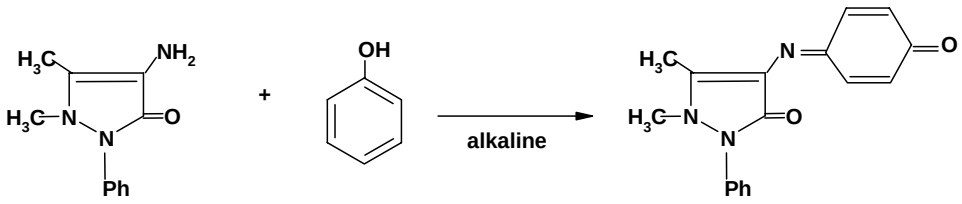


Fig 2. Coupling reaction between phenyl and 4-AAP

Optimum conditions affecting the reaction of DO.HCl and LD with 4-AAP were studied carefully. The effect of pH was studied in the pH ranged from 2-12 using universal buffer. From the data it was found that the pH of 12 was the most suitable for microdetermination of the active ingredients at λ_{max} = 475 and 454 nm for DO.HCl and LD, respectively.

Applying the molar ratio method, it was found that DO.HCl or LD interacts with 4-AAP to form product in ratio 1:1 [4-AAP]: [active ingredient] at pH = 12. By following the reaction at different time intervals (Figure 3), it is found that the suitable time needed for complete reaction

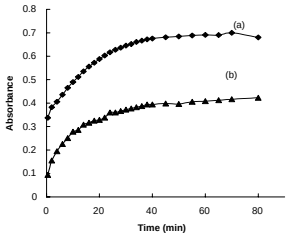


Fig. (3): Effect of time on the absorbance of (a) DO.HCl and (b) LD with 4-AAP and universal buffer of pH = 12 .

was 32 and 38 minutes. Also, it was found that maximum absorbance was attained at temperature 30 ± 2 and 38 ± 2 ^{0}C in case of DO.HCl and LD, respectively as given in Figure (4).

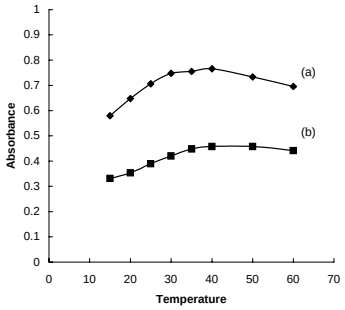


Fig. (4): Effect of temperature on the reaction of (a) DO.HCl and (b) LD with 4-AAP using universal buffer of pH = 12.

Under the optimum conditions, a correlation was obtained between absorbance (A) and the concentration (C) over the range at 37.9-170.6 and 49.3-221.8 μg mL^{-1} of DO.HCl and LD, respectively. The apparent molar absorptivity, Sandell sensitivity, standard deviation (SD) and coefficient of variation (CV) for each active ingredient were tabulated in Table (1). The apparent molar absorpitivity (ε) of the resulting coloured products were found to be 3.375x10^4 and 2.434x10^4 L. mol^{-1}.cm^{-1}, whereas Sandell sensitivities were 3.9x10^{-3} and 2.9x10^{-3} μg cm^{-2} for DO.HCl and LD, respectively. The correlation coefficient was found to be 0.999, while the SD was 0.274 and 0.262 for DO.HCl and LD, respectively. The low values of CV and SD indicated the high accuracy, precision and reproducibility of the proposed method to determine catecholamine derivatives.

Table 1. Different analytical parameters for the determination of DO.HCl and LD using 4-AAP reagent and universal buffer.

Parameters	DO.HCl	LD
Detection range (mg/L)	37.9 – 170.6	49.3 – 221.8
Correlation coefficient	0.999	0.999
Molar absorptivity $L.mol^{-1}cm^{-1}$	$3.375x10^4$	$2.434x10^4$
SD	0.274	0.262
CV (%)	0.76	0.71
Sandell Sensitivity	$3.9x10^{-3}$	$2.9x10^{-3}$
Number of replicates ν_1 , ν_2	5, 5	5,5
F-test	5.11	5.8
F*-test	5.05	5.05
Number of replicates ν_1	5	5
t-test	2.5	2.8
T*-test	4.032	4.032

F*- test: are the values for V as degree of freedom for variation confidence level.
t*-test: are the values for V as degree of freedom for 99 % confidence level.

Interference

Several pharmaceutical preparations were associated with flavoring agents, diluents and excipients. The common tolerances, which examined in our proposed procedure with active ingredients DO.HCl and LD, were glucose, acetone ascorbic acid, catechol, phenol, pyrogallol, resorcinol and hydroquinone as shown in Table (2).

Table (2): Effect of different tolerances on the determination of DO.HCl and LD using 4-AAP and universal buffer of pH=12.

Tolerant	DO.HCl		LD	
	Fold	% recovery	Fold	% recovery
-----	-----	100.0	-----	100.0
Glucose	10	100.0	10	100.5
Ascorbic acid	10	-------	10	104.0
	1	102.4	1	107.9
	0.5	99.40	0.5	101.1
Catechol	10	230.0	10	159.4
	0.5	156.7	1	132.1
Phenol	10	93.10	10	109.9
	1	97.00	1	104.6
	0.5	98.50	0.5	99.50
Pyrogallol	10	98.50	10	155.3
	1	91.90	1	133.1
Resorcinol	10	------	10	79.20
	1	------	1	85.00
Hydroquinone	10	98.80	10	73.90
	1	99.40	1	106.7
			0.5	95.40

Application

Determination of LD in pharmaceutical forms

The proposed method was applied satisfactory to the determination of LD in pharmaceutical preparation, such as Levocare drug. The determination of LD was applied with percent error 1.8 % that could be neglected (Table 3) where it present in the international acceptable range of error for pharmaceutical determination.[23, 24]

Determination of DO.HCl in pharmaceutical forms

Our proposed procedure applied on ampoule containing DO.HCl as active ingredient, as shown in Table (3). Detection of DO.HCl concentration in aliquot solutions was applied with percent error 0.3 %. The percent error was very small that could be neglected and in acceptable range of error for pharmaceutical determination.[23, 24]

On comparing the results obtained by the proposed method with those obtained by the official method[25] using t -test and for accuracy and F- test for precision[26], the calculated values of t and F tests under confidence limit 99 % = 2.5 – 2.8 and 5.11 – 5.8, respectively, indicating insignificant difference between the official and proposed methods and also referring to the robustness of the proposed procedure.

Table (3): Determination of DO.HCl and LD in pharmaceutical preparations using 4-AAP reagent and universal buffer (pH=12).

Drug	Name of preparation	[Drug], ppm Taken	[Drug], ppm Found	% Recovery	SD*	CV*	*Error%
DO.HCl	- Dopamine Fresenius	76.61	76.62	100.4 100.3	0.11 0.13	0.28 0.26	0.3
LD	- Alpha Chem. Advanced Pharmaceutical Industries Co. (ACAPI).	153.23 98. 197.19	153.71 97.0 191.9	98.2 97.3	0.13 0.11	0.46 0.32	1.8

* Number of replicates 3-5

* Average error

Conclusion

The proposed method for DO.HCl and LD estimation was advantageous over many reported methods. This may be attributed to their sensitivity, rapidity, noninterference with other ingredients usually present in pharmaceutical preparations, precision and good agreement with the official method. Hence this method could be used for the routine quality control analysis.

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