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Simple Analysis Used in Diagnosis and Follow-up of Schizophrenic Patients

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Dopamine acts as neurotransmitter in the central and peripheral sympathetic nervous system. Determination of dopamine (DO) was performed by spectrophotometric analysis depending on the formation of new colored compound. The proposed procedure was efficient in quantitative determination of DO as pure material in pharmaceutical preparations and in urine samples. DO concentration in urine sample of patient confirms the affection with schizophrenia and the proposed procedure was used to facilitate diagnosis and followup of schizophrenic patients. It is recommended to apply the proposed procedures as routine analysis in pharmaceutical companies for quality control and in analytical laboratories to diagnose and follow up schizophrenia.

1. INTRODUCTION

Dopamine and dopamine derivatives were a group of biogenic amines possessing a 3,4-dihydroxy substituted phenyl ring (Figure 1). They are considered as a type of hormones widely spread in animals and had also been detected in 44 plant families [1, 2]. 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 1950s that dopamine was recognized as a mammalian neurotransmitter in its own right but the demonstration of its nonuniform 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, for example, 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 like pyrocatechol, DO.HCl, and LD in either pure form or in its pharmaceutical formulation was suggested spectrophotometrically [9–11] and indirect kinetic spectrophotometry [12].

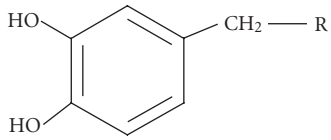
HPLC technique is most predominantly used for screening of many clinical diagnosis [13–15] and to find the difference between the measured and ordered doses of catecholamine infusion [16]. Flow injection analysis (FIA) system using tubular electrode was used in the determination of DO in pharmaceutical 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 depending on the formation of coloured complex between DO and copper sulfate and 4-amonoantipyrine (4-AAP). Different experimental conditions are carefully studied before applying Beer's law. The method was applied for determining DO in urine samples collected from schizophrenic patients and pharmaceutical forms. The results obtained are of interest and are compared with these obtained by the official method.

2. EXPERIMENTAL

2.1. Procedure

An aliquot containing 74.4–417.2 ppm of DO.HCl was transferred to 10 mL measuring flask, followed by adding 4-AAP and copper sulfate. The pH was adjusted at 10-11. The total volume was completed up to 10 mL. The mixtures were



DO; R: $CH_2CH_2(NH_2)$
LD; R: $CH(NH_2)COOH$

Figure 1: Structural formulae of the investigated dopamines.

shacked well and allowed to stand at room temperature. The pH was rechecked and the absorbance was measured at 480 nm for DO.HCl against deionized water as a blank. The calibration curve was obtained applying the same procedure using standard solutions of active ingredient.

2.2. Analysis of dosage form

DO.HCl ampoule was incubated in cold, dark bottle and away from oxygen air to prevent oxidation or decomposition.

2.3. Analysis of DO in urine samples of healthy individuals and schizophrenic patients

Urine samples were allowed to stand at room temperature before pipetting. To be sure of complete homogeneity, a rapid vortex must be done for 30 seconds.

Certain volume from urine sample was mixed with 4-AAP and copper sulfate at pH 10-11. The total volume was completed up to 10 mL measuring flask using deionized water. The method was completed under optimum conditions.

From the calibration curve, the concentration of dopamine in different urine samples can be calculated.

2.4. Results and discussion

4-AAP reacts with phenolic-type compounds according to the reaction shows in Figure 2. The reaction product may be of any color from red to purple depending on the phenolic-type compounds involved [19–21].

Optimum conditions affecting the reaction of DO.HCl with copper sulfate and 4-AAP were studied carefully. The effect of pH was studied in the pH ranging from 9 to 12 and it was obvious that the most suitable pH is 10-11 for microdetermination of DO.HCl at $\lambda_{max} = 480$ nm.

Applying the molar ratio method, it was found that DO.HCl interacts with 4-AAP and copper sulfate to form product in ratio 2 : 2 : 1 as [DO] : [4-AAP] : $[Cu^{+2}]$. The solid of this reaction is separated and characterized using different tools like elemental analysis, IR, magnetic and thermal analysis. By following the reaction at different time intervals, it is obvious that the suitable time needed for complete reaction was 10–30 minutes which was attained at room temperature.

Under the optimum conditions, a correlation was obtained between absorbance (A) and the concentration (C) over the range at 74.4–417.2 μg mL^{-1} of DO.HCl (as shown in Figure 3). 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 absorptivity (ε) of the resulting colored products was found to be 2.979×10^4 L $\cdot$ mol^{-1} $\cdot$ cm^{-1}, whereas Sandell sensitivities were 3×10^{-3} μg cm^{-2}. The correlation coefficient was found to be 0.999, while the SD was 0.06–0.3. The low values of CV and SD indicated the high accuracy, precision, and reproducibility of the proposed method to determine DO.HCl.

2.5. Interference

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

3. APPLICATIONS

3.1. Determination of DO.HCl in pharmaceutical forms

Our proposed procedure was 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 was in acceptable range of error for pharmaceutical determination [22, 23].

The calculated values of t and F tests [24] under confidence limit 95% = 2.5–2.8 and 5.11–5.8, respectively, indicated insignificant difference between the official [25] and proposed methods and also referred to the robustness of the proposed procedure.

3.2. Determination of DO in urine samples of schizophrenic patients

Before our method, schizophrenia was diagnosed by physicians clinically through an interview with the patient and followed-up schizophrenic patients via clinical diagnosis. Proper response and improvement appear clinically within 4–6 weeks from starting antipsychotic drugs [26].

In the treatment of schizophrenia, more than in many other diseases, individual patients respond differently to medication. Despite recent advances in the treatment of schizophrenia, there remains a number of unmet needs in therapy for schizophrenia management like low response, high relapse rates [27–30], nonresponse [27, 30–37], nonadherence [27, 29] or challenging road ahead.

Nowadays with our new option, analytical test is ordered after a psychiatrist performing his clinical examination [38] on the patient and suspecting that patient has schizophrenic symptoms [39]. Actually it can be used as a monitoring tool to follow up a patient treatment and in confirming psychiatrist findings. In addition, it can be applied before treatment,

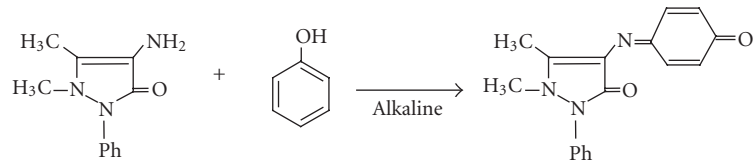


Figure 2: Coupling reaction between phenyl and 4-AAP.

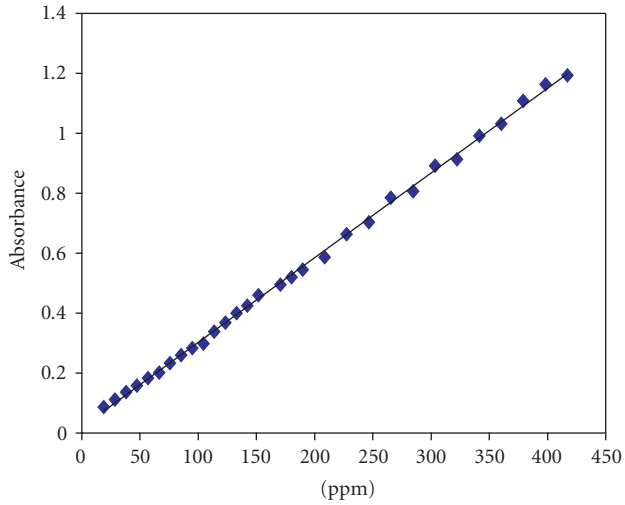


Figure 3: Calibration curve of DO with copper sulfate and 4-AAP.

after fifteen days from starting treatment and after thirty days from treatment. By this way, a psychiatrist will have facilities to evaluate the schizophrenic patient as a whole and to follow up the responsibility of the patient to recommended treatment according to the DO concentration present in urine sample. Moreover, it can be ordered as a routine analysis for early detection when a patient has a family history of schizophrenia.

3.3. Modification in spectrophotometric determination of DO in urine samples

The modification is based on formation of colored stripe sheet, which was picked from the calibration curve of DO.HCl with copper sulfate and 4-AAP under optimum conditions. The collected urine sample was mixed with copper sulfate and 4-AAP and allowed to stand under optimum conditions till complete complex formation, then the end colored product was compared with colors present in colored stripe sheet. By this way, we will have ability to detect the concentration of DO in the urine sample easily without the need to record absorbance at a certain wavelength using spectrophotometer.

So we can conclude that modification in our procedure facilitates quantitation of DO in urine sample without need to form calibration curve from time to time using standard DO material.

Table 1: Different analytical parameters for the determination of DO.HCl, using copper sulfate and 4-AAP.

Parameters	DO.HCl
Detection range (mg/L)	74.4–417.2
Correlation coefficient	0.999
Molar absorbitivity ($L \cdot mol^{-1} \cdot cm^{-1}$)	2.979×10^4
SD	0.06–0.3
CV (%)	0.1–0.12
Sandell sensitivity	3×10^{-3}

Table 2: Effect of different tolerants on the determination of DO.HCl using copper sulfate and 4-AAP.

Tolerant	Fold	Recovery %
—	—	100.0
Glucose	10	100.7
Acetone	10	99.30
Ascorbic acid	10	—
	1	104.1
	0.5	102.6
Urea	10	97.85
	1	98.90
	0.5	99.60
Catechol	10	370.8
	0.5	186.7
Phenol	10	808.8
	0.5	189.3
Pyrogallol	10	138.7
	1	113.7
	0.5	107.4
Resorcinol	10	374.9
	1	93.40
	0.5	95.94
Hydroquinone	10	239.8
	1	161.6
	0.5	136.5

4. CONCLUSION

The proposed method and its modification for DO estimation were advantageous over many reported methods, where they facilitate quantitation of DO in urine samples without the need to form calibration curve from time to time using standard DO material. Moreover, early detection, diagnosis, followsups and prevention of relapse of schizophrenic patients will be fast and easy.

This method could be used for the routine quality control analysis due to its sensitivity, rapidity, noninterference

Table 3: Determination of DO.HCl in pharmaceutical preparation using copper sulfate and 4-AAP reagents.

Drug	Name of preparation	Drug, (ppm)		Recovery %		SD		*t test	F test
		Taken	Found	PM	OM	PM	OM		
DO.HCI	Dopamine Fresenius	76.61	77.34	100.9	100.8	0.10	0.21	1.18	4.41
		153.23	153.58	100.2	99.17	0.11	0.25	1.05	5.16
	Dopamine Pierre Faba	76.61	78.05	101.8	102.2	0.17	0.20	1.1	1.38
		153.23	153.58	100.2	101.1	0.13	0.17	1.5	1.71

*t test shows the values for V as degree of freedom for 95% confidence level (number of replicates $\nu_1 = 5$).

*F test shows the values for V as degree of freedom for variation confidence level (number of replicates ν_1,, $\nu_2 = 5, 3$).

with other ingredients usually present in pharmaceutical preparations, precision, and good agreement with the official method.

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