RESEARCH PAPER

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Rapid Spectrophotometric Determination of Dopamine hydrochloride with Chloramine-T

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A rapid, sensitive and simple spectrophotometric method is developed for the determination of dopamine hydrochloride (DPH) using sodium salt of N-chloro-4-methylbenzenesulphonamide (chloramine-T or CAT) and traces of Copper (II) in a buffer medium of pH 7. Beer's law is obeyed in the range of DPH concentrations of 2-20 µg/ml at the maximum absorption of 490 nm. The method is successfully employed for the determination of DPH in pharmaceutical preparations.

SASTRY et al[1] have reported the determination of DPH using p-aminoacetophenone in presence of sodium metaperiodate, the Sandell's sensitivity and molar absorptivity were reported as 0.066 µg/cm^2 and 2.85x10^3 l/mol/cm respectively. The same authors had reported that CAT is less effective. Although, the reaction of DPH with CAT is slow in neutral medium, addition of copper sulphate makes the reaction almost instantaneous.

The present work describes the reaction of DPH with chloramine-T (CAT) in presence of copper (II) which acts as a catalyst at a pH of 7 yielding a red chromogen with an absorption maximum at 490 nm. The method offers the advantages of rapidity, sensitivity and simplicity without the need for extraction or heating.

EXPERIMENTAL

Instrument :

A JASCO Model Uvidec-610 UV-Vis spectrophotometer with 1.0 cm matched cells was used for absorbance measurements. The pH measurements were made with an Elico digital pH meter.

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Standard, sample and reagent solution :

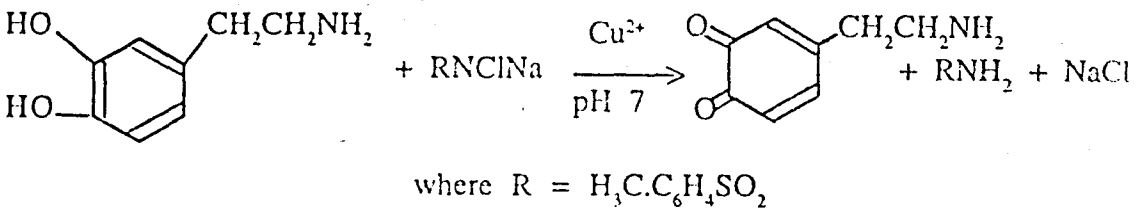
DPH (TTK Pharma, India) solution was freshly prepared by dissolving 100 mg of DPH in 100 ml of water. DPH solutions of lower concentrations (100 µg/ml) were prepared by suitably diluting the stock solution. The DPH injection is suitably diluted to get the required concentration of the drug. CAT solution (0.5%) was prepared by dissolving 0.5 g of CAT in water and diluting to 100 ml with water. A 0.001 M copper(II) solution was prepared by dissolving the requisite amount of copper sulphate in 100 ml of water. Buffer[2] of pH 7 was prepared by mixing 39 ml of sodium hydrogenphosphate (0.2 M) and 61 ml of disodium hydrogenphosphate (0.2 M) and diluting to 200 ml.

About 50-700 µl of standard solution of DPH was transferred into a 25 ml calibrated flask. 0.5 ml of buffer solution, 1.5 ml of copper sulphate solution and 0.5 ml of CAT solution were added and the mixture was set aside for 5 min. The contents were diluted to the mark with water and mixed well. The absorbance was measured at 490 nm against a corresponding reagent blank. DPH injection solutions were appropriately diluted with water to get the required concentration of the drug and the above procedure was repeated. The amount of DPH was calculated from a calibration graph.

Table 1. Determination of DPH in pharmaceutical preparations

Sample	Label claim (mg/5ml)	DPH found/5ml Recovery ± RSD[a]		
		Proposed method	USP method(5)	Metaperiodate method(3)
Injection 1	200	200.8±0.54	201±0.76	201±0.93
Injection 2	200	201±0.46	201.5±0.66	201.7±0.85

[a]Average of six determinations
[1]Marketed by TTK pharma
[2]Marketed by TRIOKA parenterals



Scheme 1: Oxidation of dopamine by Chloramine-T

RESULTS AND DISCUSSION

A red coloured oxidation product with an absorption maximum at 490 nm is formed when DPH is allowed to react with CAT in presence of copper(II) ion in a buffer medium of pH 7. Beer's law holds good over the range 2-20 µg/ml. The optimum photometric range from Ringbom's plot is found to be between 5-16 µg/ml. Molar absorptivity and Sandell's sensitivity of the reaction as calculated from the Beer's law data is 3.02 x 10^3 l/mol/cm and 0.0507 µg/cm^2 respectively. The minimum detection limit of the proposed method is 0.2 µg/ml. The reproducibility of the method was assessed by carrying out ten replicate analyses of a solution containing 350 µg of DPH solution in a final volume of 25 ml. The relative error and relative standard deviation were found to be ± 0.75 % and 0.011 respectively. Using the linear least squares treatment, the values of slope (a), intercept (b) and correlation coefficient (r) were found to be 0.0133, 0.0156 and 0.998 respectively. The concentration of DPH can be calculated from the regression equation y = ax + b, where x is the concentration of DPH in µg/ml.

The oxidation of DPH by CAT alone is very slow. In presence of traces of Cu(II) ion, the oxidation is almost instantaneous to yield a red chromogen. The reaction is shown in Scheme 1. The oxidized product is less stable (5 min), in the absence of buffer, but in the presence of buffer of pH 7, the stability of the coloured product was enhanced to 50 min. The order of addition of reagents is followed as mentioned in the procedure. It was found that a 0.5% concentration of CAT in the range 0.25-1 ml, a 0.001 M concentration of copper sulphate in the range 1-2.5 ml, and the buffer solution in the range 0.2-1 ml was necessary for the achievement of maximum colour intensity.

Sastry et al[1,3] and El-Kommos et al[4] have discussed the oxidation of catechol amines forming an O-benzoquinone product. In the present investigations, CAT is oxidizing DPH in presence of cu(II) ions and the latter function as a catalyst. The oxidation product obtained by the present method and by the methods mentioned above[1,3,4] were detected by tlc using a mixture of methanol and ammonia as mobile phase indicating the product as substituted O-benzoquinone. The colour reaction does not

seem to depend only on the catechol function, because pyrocatechol gives a violet colour by the proposed method, indicating that-$CH_2CH_2NH_2$ group in DPH is responsible for the red colouration in the present method.

An attractive feature of the method is its relative freedom from interference by the usual excipients such as sodium chloride, starch, magnesium stearate, gum acacia and sodium metabisulphate. While, vitamin-C, levodopa, methyldopa, adrenaline and catechol were found to interfere. The applicability of the method to the assay of pharmaceutical preparations was examined. The results of the assay of DPH injections presented in Table 1 compare favourably with the quoted values and those obtained by the official method of USP[5]. The results were cross-checked by the reported method[3].

In conclusion, the proposed method is economical, simple, rapid and sensitive. The use of CAT as an analytical reagent along with Cu(II) as catalyst provides a fairly high sensitivity compared with most of the other reagents for the determination of DPH.

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