Spectrophotometric Determination of Certain Vicinal Dihydroxybenzene Derivatives with Isoniazid

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A simple sensitive and accurate spectrophotometric method is developed for the determination of vicinal dihydroxybenzene derivatives [pyrocatechol (PCL), dopamine (DPH), levodopa (LDP) and methyldopa (MDP)] using pyridine-4-carboxylic hydrazid (isoniazid) in alkaline medium. Beer's law of the coloured species is obeyed in the range 1.2 to 12 µg/ml at the maximum absorption of 480 nm. The molar absorptivity found to be 1.4312 x 10^4, 1.5015 x 10^4, 1.0924x10^4 and 7.2163x10^3 l/mol/cm for DPH, PCL, LDP and MDP respectively. The proposed method is successfully applied for the determination of DPH, LDP and MDP in injections and tablets of pharmaceutical preparation. The reliability of these methods is established by parallel determination with the reported and official methods.

Dopamine hydrochloride [4-(2-Aminoethyl)-pyrocatechol], DPH, methyldopa [3-(3,4-dihydroxyphenyl)-2-methylalanine], MDP, levodopa [2-amino-3-(3,4-dihydroxyphenyl)-propanoic aid], LDP and pyrocatechol, PCL are vicinal dihydroxybenzene derivatives in which either the 3-or 4-position is unsubstituted and these positions are not sterically blocked. Various methods like spectrofluorimetry[1,2], spectrophotometry[3], ion-exchange column chromatography[4], gas chromatography[5,6] and radioimmunoassay[7,8] have been described in the literature for the determination of dopamine and dopa from the biological samples and pharmaceutical preparations. Most of these methods lack the simplicity needed for routine analysis.

In our previous publication[9], vicinal dihydroxybenzene derivatives were estimated by oxidizing with N-bromosuccinimide (NBS) followed by oxidative coupling with isoniazid (INH) in basic medium to form a red coloured product having maximum absorbance between 480-490 nm. However, in the present work, it is estimated with INH by heating instead of adding NBS as an oxidizing agent. The method is simple, accurate and sensitive.

A Shimadzu Double-Beam Spectrophotometer UV-150-02 with 1.0 cm matched glass cells were used for the spectral measurements.

DPH (Sigma, USA), levodopa (SD Fine, India), methyldopa (SD Fine, India) and pyrocatechol (CDH, India) solutions were freshly prepared by dissolving 100 mg of each in 100 ml of water separately. The solutions of lower concentration (100 µg/ml) were prepared by suitably diluting the stock solutions. The DPH injection is suitably diluted to get the 100 µg/ml of the drug. Twenty tablets were weighed and finely powdered. A weighed amount of the powder equivalent to 50 mg of LDP or MDP was dissolved in 25 ml of water and filtered using Whatman filter paper. The filtrate was made up to 100 ml and an aliquot of this solutions diluted to get the required concentration of the drug. 1.0% INH and 0.1 M sodium hydroxide solution were prepared by dissolving suitable amounts of INH and sodium hydroxide in water.

Aliquots of standard solutions of PCL (6.25-137.5 µg), DPH (25-265 µg), LDP (25-250 µg) or MDP (25-300 µg) were transferred to a 25 ml calibrated flask. To each flask 3.5 ml of 1.0% INH and 1.5 ml of 0.1 M sodium hydroxide solution were added. The mixture was heated in boiling water bath, then cooled to room temperature. The contents were diluted to the mark with water and mixed well. The absorbance was measured at 480 nm against a reagent blank.

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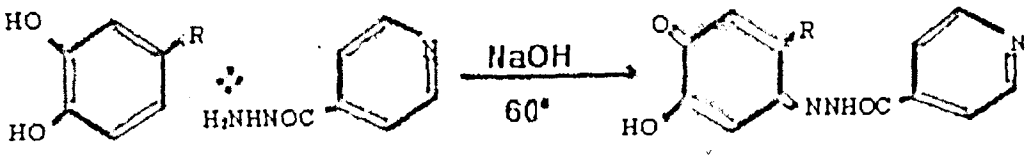

Where R = H: pyrocatechol
R = $CH_2CH_2NH_2$: dopamine
R = $CH_2C(CH_3)(NH_2)$ COOH : methyldopa
R = $CH_2CH(CH_2)COOH$: levodopa

SCHEME 1

Table 1 - Optical characteristics and statistical parameters of vicinal dihydroxybenzene derivatives in the proposed method

Optical character	Values of			
	PCL	DPH	LDP	MDP
Beer's law range (µg/ml)	0.25-5.5	1.0-10.6	1.0-10.0	1.0-12.0
Optical photometric range (µg/ml)	1.0-5.0	1.5-9.5	1.9-9.5	3.0-10.6
Molar absorptivity (1/mol/cm)	$1.5015x10^4$	$1.4312x10^4$	$1.0924x10^4$	$7.2163x10^3$
Sandel's sensitivity (µg/cm^2)	0.0073	0.0133	0.0181	0.0293
Regression equation (y)*				
Slope (a)	0.1396	0.0666	0.0511	0.0341
Intercept (b)	0.0032	0.0327	0.0273	0.0115
Correlation coefficient (r)	0.9963	0.9994	0.998	0.9896
RSD (%)#	1.2	2.8	2.0	1.7
Relative error	±0.70	±0.9	±1.2	±1.0
Stability of the red coloured product (h)	15.0	2.5	1.5	2.0

* y = ax+b, where 'x' is the concentration of PCL, DPH, LDP or MDP in µg/ml. # Relative standard deviation (n=6).

A characteristic red coloured product is formed when INH is allowed to react with vicinal dihydroxybenzene derivatives (VDD) in presence of sodium hydroxide in aqueous medium. It takes about 1 h at 27±3° to complete the reaction. If the reaction mixture is heated to about 60°, red colour product is formed immediately. The details of optical characteristics and statistical parameters of VDD are summarized in Table 1. The reproducibility of the method was assessed by carrying out ten replicate analyses of solutions containing 150 µg/ml of VDD in a final volume of 25 ml.

VDD itself oxidizes at higher temperature without any oxidizing agents to give o-benzoquinone. The literature reflected that aryl hydrazines are easily oxidized by benzoquinone to give aryldiimide and hydroquinone[10-12]. INH by virtue of its strong electron-donating group, couples with o-benzoquinone derivative in alkaline medium leading to the formation of red coloured oxidative coupled products at about 60° (Scheme 1)[9,13]. The resultant products of the proposed method are studied at different temperatures. The results indicate that the absorbance values remain constant in the temperature range 0-70°. At

Table 2 - Determination of vicinal dihydroxybenzene derivatives in pharmaceutical preparation

Sample	Label amount in mg	% Recovery*±RSD		
		BP method	Reported method[12]	Proposed method
DPH				
Injection[a]	200/5 ml	98.85±1.25	99.04±1.10	99.92±0.51
Injection[b]	200/5 ml	99.20±0.82	100.09±0.99	100.10±0.75
Tablelts				
LDP[c]	500	98.78±1.10	97.83±0.96	99.84±0.90
MDP[d]	250	99.50±0.86	-	99.98±1.02

*Average of six determinations. [a]Marketed by TTK Pharma, [b]Marketed by TRIOKA Parenterals, [c]Marketed by Wallace, [d]Marketed Merind Limited.

higher temperature the absorbance values decrease, indicating the dissociation of the products on prolonged heating.

It was found that a 1.0% concentration of INH in the range 3.0-4.0 ml and 0.1M concentration of sodium hydroxide in the range 1.0 to 2.5 ml were necessary for the achievement of maximum colour intensity.

An antioxidant, sodium metabisulphate and sodium chloride that is commonly present in the DPH injection and also commonly used excipients such as starch, talc, glucose, lactose, dextrose and magnesium stearate, did not interfere, while Vitamin-C, adrenaline and noradrenaline were found to be interfered in the proposed method.

The proposed method was applied to assay pharmaceutical preparations containing above VDD. The results obtained (Table 2) compared favourably with the reported[12] and the official method[14]. The proposed method is simple, precise, sensitive and accurate. This method can be successfully applied as an alternative to the existing methods.

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REFERENCES

1. Bell, C.E. and Somerville, A.R., **Biochem. J.,** 1996, 1C, 98.
2. Imai and Kazuhizo, **J. Chromatogr.,** 1995, 135, 105.
3. Sane, R.T., Deshpande, P.M., Sawant, C.L., Dolas, S.M., Nayak, V.G., and Zarapkar, S.S., **Indian drugs,** 1987, 24, 199.
4. Seki, Tokaichizo, Wada and Hiroshi, **J. Chromatogr.,** 1975, 114, 227.
5. Murphy, P.J., William, T.L. and Kau, D.L., **J. Pharmacol. Exp. Ther.,** 1976, 199, 423.
6. Satoshi, K. and Zenzo, T., **Chem. Pharm. Bull. Tokyo,** 1968, 16, 1091.
7. Erlanger, B.F., **Pharmacol. Rev.,** 1973, 25, 271.
8. Ricebery, L.J., Vunakis, H.V. and Levin, L., **Anal. Biohcem.,** 1974, 60, 551.
9. Nagaraja, P, Srinivasa Murthy, K.C., Rangappa K.S. and Made Gowda, N.M., **Talanta,** 1998, 46, 39.
10. Hans-Dieter, Patai, S., **The Chemistry of Quinonoid compound,** Part 1, John wiley, London, 1974, 403.
11. Mahfouz, N.M.A. and Emara, **Talanta,** 1993, 40, 1023.
12. El-Kommos, M.E., Mohamed, F.A., and Khedr, A.S., **J. Assoc. Off. Anal. Chem.,** 1990, 73, 516.
13. Sastry, C.S.P., Das, V.G. and Rao, K.E., **Analyst,** 1985, 110, 395.
14. **British Pharmacopeia,** London, SIN 85 NQ, 1993, 239, 380 and 424.