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Spectrophotometric and Liquid Chromatographic Determination of Dopamine from Pharmaceutical Preparations Using 2-Hydroxynaphthaldehyde as Derivatizing Reagent

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Dopamine has been determined by spectrophotometry and liquid chromatography as derivative of 2-hydroxynaphthaldehyde. The optimal derivatization occurred at pH 8 with enhancement in spectrophotometric sensitivity. The spectrophotometry was used for the analysis of dopamine in pharmaceutical preparations. The liquid chromatography of dopamine was carried out in the presence of 4-aminobutyric acid and tyramine. The separation was obtained from Phenomenex C-18 column 5 µm (150 × 4.6 mm id) by isocratic elution with methanol:acetonitrile: water (54:4:42 v/v/v) with a flow rate of 1.2 mL min^{-1}, UV detection was at 330nm. Linear calibration curves were obtained for each within 0.33-40.00 µgmL^{-1} with detection limits in the range 1.9-8.2 ng injection^{-1} (10 µL). The dopamine was determined in pharmaceutical preparations with coefficient of variation within 0.8-2.5 %.

Key Words: Dopamine, 2-Hydroxynaphthaldehyde, HPLC, Pharmaceutical analysis.

INTRODUCTION

Dopamine (DP) is an important biological active compound and is involved in autonomic neurochemical transmission. It is a member of catecholamines. The compounds tyramine and 4-aminobutyric acid have definite role in the biological system[1,2]. Dopamine could be determined by liquid chromatography[3-11], gas chromatography[9-16], electro-analytical techniques[14-20], flow injection[18,22], capillary electrophoresis[20-29], mass spectrometry[30], fluorimetry[31] and spectrophotometry[32,33]. Liquid chromatography connected with electrochemical[34,35], spectrofluori-metric[36,37] or chemiluminescence's detection[38] is more frequently used. The reagents used for pre-column derivatization for spectrofluorimetric detection are *ortho*-phthaldehyde[39], ethylenediamine, 1,2-diphenylethylenediamine[40], hydroxy-succinimidyl 3-indolylacetate[41], dansyl chloride[42,43], trinitrobenzene

sulphonic acid[44] and N-hydroxysuccinimidyl fluorescin *o*-acetate[45]. High performance liquid chromatography connected with ultraviolet detection is more frequently available. It was therefore an attempt was made to analyze dopamine using inexpensive derivatizing reagent with short analysis time with isocratic elution and UV detection. The stable signals obtained could be of value in analytical determination of dopamine. The reagent 2-hydroxynaphthaldehyde has resembling structure to naphthalene-2,3-dicarboxaldehyde (Fig. 1) and has been used for the HPLC determination of 4-aminobutyric acid[46], glutamine[47], tryptophan, histidine and methionine[48,49]. The work examines the use of 2-hydroxynaphthaldehyde for the determination of dopamine together with 4-aminobutyric acid and tyramine.

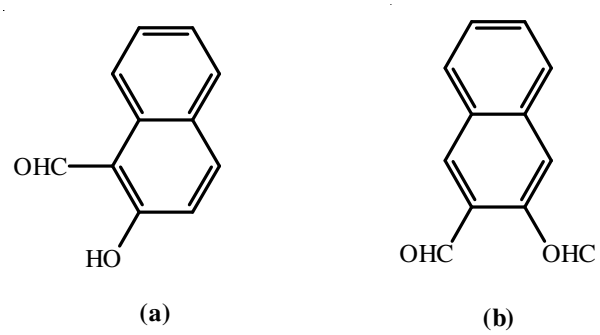

Fig. 1. Structural formula of (a) 2-Hydroxy-1-naphthaldehyde (HN) and (b) Naphthalene-2,3-dicarboxaldehyde

EXPERIMENTAL

Dopamine hydrochloride, tyramine, 4-aminobutyric acid and methanol (E. Merck, Germany) and acetonitrile (Fluka) were used. Freshly prepared double distilled water from all glass was used for HPLC elution. Hydrochloric acid (37 %), potassium chloride, acetic acid, sodium acetate, ammonium acetate, sodium borate, sodium bicarbonate, sodium carbonate, ammonium chloride and ammonia solution (23 %) were from (E. Merck). Glycine, serine, leucine, valine, arginine (E. Merck), phenylalanine, tyrosine, histadine, aspargine, lysine, isolucine (Fluka), ascrorbic acid, uric acid, citric acid and sodium citrate(E. Merck) were used.

Buffer solutions within pH 1-10 at unit pH interval were prepared from the following; hydrochloric acid (0.25 M), potassium chloride (0.25 M), acetic acid (0.25 M), sodium acetate (0.25 M), ammonium acetate (0.25 M) and sodium borate (0.25 M), sodium bicarbonate (0.25 M), sodium carbonate (saturated), ammonium chloride (0.25 M) and ammonia (0.25

M). The pH measurement was made with Orion 420A pH meter with combined glass electrode and reference internal electrode. Spectrophotometric studies were carried out with double beam Hitachi 220 Spectrophotometer (Hitachi (Pvt.) Ltd, Tokyo, Japan) with 1cm dual silica cuvettes. HPLC studies were carried out with Hitachi 655A liquid chromatograph combined with variable wavelength UV monitor, Rheoydne 7125 injector and Hitachi recorder 056. Column Phenomenex, C-18, 5 μm (150 × 4.6 mm id) (Torrance, CA, USA) was used throughout the study.

Analytical procedures

Spectrophotometric: Solution (0.1-1 mL) containing 15-153 μg dopamine, 10-50 μg 4-aminobutyric acid or 13.7-68.5 μg tyramine was transferred separately in 5 mL volumetric flask and were added 0.4 mL of borax buffer pH 8 and 0.6 mL reagent HN (0.3 % w/v in methanol). The mixture was heated for 5 min at 75ºC and after cooling at room temperature volume was adjusted to the mark with methanol. The solutions were mixed well and the absorbance was measured at 417 nm for 4-aminobutyric acid, 415 nm for tyramine and 422 nm for dopamine against reagent blank prepared by following same procedure but addition of dopamine, tyramine or 4-aminobutyric acid was omitted.

Determination of dopamine from pharmaceutical preparations: Solution (0.2 mL) from dopamine injection (5 mL) (Abbot Lab. (Pvt.) Karachi) and Intropin Dupont (Knoll Pharmaceuticals Ltd. Korangi, Karachi) was transferred to 10 mL volumetric flask and volume was adjusted with methanol:water (1:1 v/v). The solution 0.2 mL was transferred to 5mL flask and procedure 2.2. (I) was followed. The amount of DP in injections was evaluated from standard calibration curve.

HPLC Procedure: Solution (0.2-1.0 mL) containing 4-aminobutyric acid (1.65-41.2 μg), dopamine (7.0 -39.0 μg) and tyramine (12.5-173.5 μg) was transferred to 5 mL volumetric flask and procedure was followed as 2.2 (I). The solution was mixed well and 10 μL was injected on the column Phenomenex C-18 (150 × 4.6 mm id) and derivatives were eluted with a ternary mixture of methanol:acetonitrile:water (54:4:42 v/v/v) with a flow rate of 1.2 mL min^{-1} and UV detection was at 330nm.

Analysis of dopamine in pharmaceutical preparations: Solution (1 mL) from dopamine injection (5 mL) (Abbot Lab. (PVT), Karachi) and Intropin Dupont (Knoll Pharmaceuticals Ltd, Korangi, Karachi) were diluted to 5 mL. The solution (0.1 mL) was taken and volume was adjusted to 1 mL. The analytical procedure 2.2. (III) was then followed and the amount of dopamine in injection was evaluated from external calibration curve.

RESULTS AND DISCUSSION

The reagent 2-hydroxynaphthaldehyde reacts with amino group containing compounds to form highly stable Schiff bases. The reactions were examined towards dopamine, 4-aminobutyric acid and tyramine (Fig. 2). The reactions were initially monitored spectrophotometrically to optimize the reaction conditions for dopamine. The conditions which gave maximum responses were considered as optimal. The pH was varied within the range 1-10 at unit interval. The reaction was observed within pH 4 -10 and maximum at pH 8 (Fig. 3). The borax buffer used covered the pH range satisfactorily. Heating time at 75ºC was varied from 2.5 to 15 min. The reaction of dopamine with 2-hydroxynaphthaldehyde was fairly rapid and indicated maximum absorbance within 5 min and remained constant up to 10 min. The heating time of 5 min was selected. The amount of derivatizing reagent was varied between 0.2 to 1.0 mL at an interval of 0.2 mL with 2-hydroxynaphthaldehyde concentration of 0.3 % w/v in methanol. The optimal response was obtained with 0.6 mL.

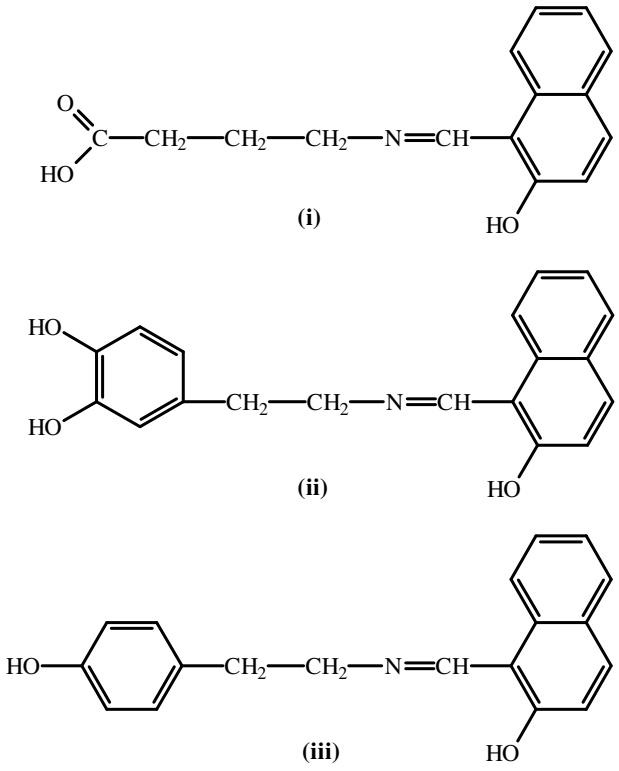

Fig. 2. Structural formula (I) 4-Aminobutyric acid-2-hydroxynaphthaldehyde (II) Dopamine-2-hydroxynaphthaldehyde and (III) Tyramine-2-hydroxy-naphthaldehyde

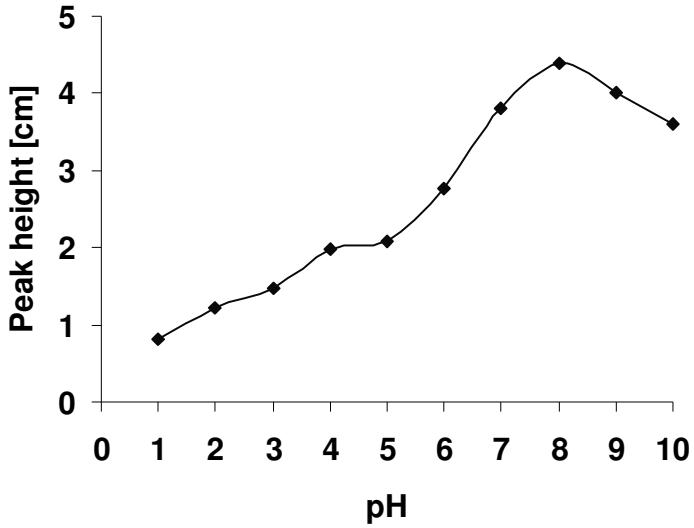

Fig. 3. Effect of pH on the absorbance of 2-hydroxynaphthaldehyde-dopamine derivative

At optimized conditions dopamine with 2-hydroxynaphthaldehyde indicated molar absorptivity 27000 L mol^{-1} cm^{-1} at 422 nm and obeyed Lambert-Beer's law with 3.5-34.7 μg mL^{-1} with coefficient of determination $r^2 = 0.9991$ and the regression equation was Y = 0.0252x. The spectrophotometric data of reactions of 2-hydroxynaphthaldehyde with tyramine and 4-aminobutyric acid is also summarized in (Table-1). All the derivatives were observed highly stable and did not show any change in absorbance up to 24 h. The spectrophotometric procedures were examined for the determination of dopamine from two pharmaceutical preparations. The results were obtained with relative deviation of 0.5-2.6 % from labeled values with C.V 1.4 -2.6 %. The presence of HCl, sodium acetate, citric acid and sodium citrate in the pharmaceutical preparation did not affect the determination.

TABLE-1
QUANTITATIVE SPECTROPHOTOMETRIC DATA OF HN DERIVATIVES IN METHANOL-WATER

Name of derivative	Wavelength (nm)	Molar absorptivity (L mol^{-1} cm^{-1})
Dopamine–2-hydroxynaphthaldehyde	422	27000
4-Aminobutyric acid–2-hydroxy-naphthaldehyde	412	30000
Tyramine–2-hydroxynaphthaldehyde	418	18881

Spectrophotometric procedure indicated high sensitivity but 2-hydroxynaphthaldehyde derivatives of dopamine, tyramine and 4-aminobutyric acid indicated maximum absorbance within same region and the absorption bands overlapped each other significantly, it was therefore considered necessarily to separate the derivatives before spectrophotometric detection. An attempt was made to develop reversed phase HPLC method for the determination of dopamine by UV detection. The derivatives of dopamine, 4-aminobutyric acid and tyramine separated completed from each other and from derivatizing reagent 2-hydroxynaphthaldehyde, when eluted from Phenomenex C-18 (150 × 4.6 mm id) column with mixture of methanol:acetonitrile:water (54:4:42 v/v/v/) with a flow of 1.2 mL^{-1}. The 2-hydroxynaphthaldehyde derivatives contain naphthyl and phenyl chromophoric groups which absorb strongly within UV region. The wavelength of UV monitor was varied from 250-350 nm at an interval of 10 nm. Better response was observed at 330 nm and was selected. The (Rs) between adjacent peaks was > 2.2, with analysis time of 8 min. Inter and intra day variation in terms of average peak height and retention time (n = 5) were obtained with CV 1.5 - 3.2 %. The calibration curve for 4-aminobutyric acid, dopamine and tyramine were linear in the range 0.33-8.2 μg mL^{-1}, 1.4-7.8 μgmL^{-1} and 2.5-34.7 μgmL^{-1}, respectively by plotting average peak height (n = 3) *vs.* concentration. The coefficients of determinations (r^2) were observed 0.998, 0.999 and 0.996 for 4-aminobutyric acid, dopamine and tyramine, respectively. The regression equation for dopamine was Y = 0.1823x. The detection limits measured as signal to noise ratio 3:1 were 1.9, 8.24 and 6.94 ng $injection^{-1}$ (10 μL) for 4-aminobutyric acid, dopamine and tyramine, respectively.

The test solutions (n = 4) of dopamine were analyzed by using 2-hydroxynaphthaldehyde as derivatizing reagent and relative % errors were observed within ± 0.6-2.8%.

The effect of different amino acids on the determination of dopamine was examined using the derivatizing reagent 2-hydroxynaphthaldehyde. The amino acids glycine, phenylanaline, tryptophan, serine, leucine, valine, arginine, tyrosine, L-histadine, L-spargine, arginine, L-lysine and isolucine, ascorbic acid and uric acid when added at the same concentration as that of dopamine (5.12 μg mL^{-1}) did not affect the determination of dopamine.

Two dopamine injections (Dopamine hydrochloride and Intropin Dupont) were analyzed for contents of dopamine. An aliquot of sample after appropriate dilution was subjected to derivatization procedure and amount of dopamine was evaluated from external calibration. The results (Table-2) indicated C.V. of 0.8-2.4 %.

TABLE-2
ANALYSIS OF PHARMACEUTICAL PREPARATIONS BY HPLC USING HN AS DERIVATIZING REAGENT

Name of preparations	Compounds present	Amount reported (mg mL^{-1})	Amount found (mg mL^{-1}) (CV %)	RD (%)
Dopamine HCl	Dopamine HCl, citric acid, sodium citrate buffer	40	38.5 (2.5)	3.75
Intropin dupont	Dopamine HCl	40	39.1 (1.3)	2.25

Conclusion

Simple analytical procedures using spectrophotometer and HPLC connected with UV detection have been described for the determination of dopamine in the presence of 4-aminobutyric acid and tyramine using 2-hydroxynaphthaldehyde as derivatizing reagent. The detection limits obtained were 1.9 to 8 ng $injection^{-1}$. The method could be used for the determination of dopamine from pharmaceutical preparations.

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