ORIGINAL ARTICLE

SPECTROPHOTOMETRIC AND LIQUID CHROMATOGRAPHIC DETERMINATION OF DOPAMINE FROM PHARMACEUTICAL PREPARATIONS USING ACETYL ACETONE AS DERIVATIZING REAGENT

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ABSTRACT

Dopamine (DA) has been determined by spectrophotometry and by liquid chromatography as derivatives of acetylacetone (AA). The liquid chromatography of DA was carried out in the presence of hydroxyproline (HP), octopamine (OP) and tyramine (TY). The separation was obtained from Phenomenex C-18 column 5pm (150 x 4.6 mm id) by isocratic elution with methanol: acetonitrile: water (45: 6: 49*v/v/v*), UV detection was at 320nm. Linear calibration curves were obtained for each with 2.5-40.0µgml^{-1} with detection limits in the range 1.8-80ng injection^{-1} (10µl). The dopamine was determined in pharmaceutical preparations with coefficient of variation (CV) within 0.7-1.5%.

Keywords: Dopamine, spectrophotometry, liquid chromatography, derivatizing reagent.

INTRODUCTION

Dopamine (DA) is an important biological active compound and is involved in autonomic neurochemical transmission it is also a member of catecholamines. The compounds hydroxyproline (HP), octopamine (OP) and tyramine (TY) have definite role in the biological system (Thomas, 1992). DA could be determined by liquid chromatography (LC) (Bielavska and Kassa, 2000; Vander *et al*., 1989; Vuorensola *et al*., 2003), gas chromatography (GC) (Uchimura and Matsumoto, 1983; Musshoff *et al*., 2000 and Mursshoff *et al*., 2003), electro-analytical techniques (Wang *et al*., 2002 and Zhang *et al*., 2004), flow injection (Nalewaja *et al*., 2004), capillary electrophoresis (Qian *et al*., 1999 and Zhang *et al*., 2003), mass spectrometry (Musshoff *et al*., 2000), fluorimetric (Wang *et al*., 2003) and spectrophotometry (Markovic and Amrain, 1990; Nagaraja *et al*., 1998). Liquid chromatography connected with electrochemical (Bielavska and Kassa, 2000; Vander Hoorn *et al*., 1989; Vuorensola *et al*., 2003), spectrofluorimetric (Vander Hoorn *et al*., 1989 and Kawasaki *et al*., 1989) or chemiluminescence (Ragab *et al*., 2000) detection is commonly used. The reagents used for pre-column derivatization for spectrofluorimetric detection are ortho-phthaldehyde (Farrant *et al*., 1987), 1,2-diphenyl-ethylenediamine (Nohta *et al*., 1986), hydroxysuccinimidyl 3-indolylacetate (Wang *et al*., 1999), dansylchloride (Tsuchiya *et al*., 1986) and N-hydroxysuccinimidyl fluorescin-o-acetate (Wang *et al*., 2000). High performance liquid chromatography (HPLC) connected with ultraviolet (UV) detection is more frequently available, therefore an attempt was made to analyze dopamine (DA) using inexpensive derivatizing reagent with short analysis time with isocratic elution and UV detection. It was expected that the stable signals obtained could be of value in analytical determination of dopamine. The work examines the use of acetylacetone (AA) for the determination of DA together with hydroxyproline (HP), octopamine (OP) and tyramine (TY). The spectrophotometric data collected on derivatizing reactions, was used to optimize LC separation and determination. The review of literature shows that AA has not been used for the determination of DA.

EXPERIMENTAL

Chemicals and equipments

Dopamine hydrochloride, HP, OP, TY and methanol (MERCK Germany) and acetonitrile (Fluka) were used. Freshly prepared double distilled water from all glass was used for HPLC elution. G.R-grade hydrochloric acid (37%), potassium chloride, acetic acid, sodium acetate, ammonium acetate, sodium bicarbonate, sodium carbonate, ammonium chloride and ammonia (23%) were from E. Merck. Glycine, serine, leucine, valine, arginine (E. Merck), phenylanaline, tryptophan, tyrosine, L-histadine, L-spargine, arginine, L-lysine, isolucine (Fluka), ascorbic 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 (0.1M), potassium chloride (1M), acetic acid (1M), sodium acetate (1M), ammonium acetate (1M), sodium bicarbonate (1 M), sodium carbonate (saturated), ammonium chloride (1M) and ammonia (1M). 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 1 cm silica cuvettes. HPLC studies were carried out with Hitachi 655A liquid chromatograph combined with variable wavelength UV

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monitor, Rheoydne 7125 injector and Hitachi chromato-integretor 2500. Column Phenomenex, C-18, 5i~tm (150x 4.6mm id) (Torrance, CA, USA) was used throughout the study.

ANALYTICAL PROCEDURES

Spectrophotometric

To the solution containing 22.5-227.Sjtg DA, HP, OP or TY separately were added 1.5mL of reagent AA (0.4%v/v in water) and 0.4m1 carbonate buffer pH 9 in 5 ml volumetric flasks. The mixture was heated on water bath at 75ºC for 20mm and allowed to cool. The volume was adjusted to the mark with methanol. The absorption spectra were recorded against reagent blank within 250-450nm and absorbance was measured at 318nm, 322nm, 31 6nm or 31 8nm for DA, HP, OP or TY derivatives respectively. The reagent blank was prepared following same procedure except addition of DA, HP, OP or TY was omitted.

Spectrophotometric determination of dopamine from pharmaceutical preparations

Solution (0.2m1) from dopamine injection (5ml) (Abbot Lab. (Pvt.) Karachi, Pak.) and intropin dupont (Knoll Pharmaceuticals Ltd. Korangi, Karachi) was transferred to l0ml volumetric flask and volume was adjusted with methanol: water (1: lv/v). The solution 0.2ml was transferred to 5m1 flask and procedure (I) was followed. The amount of DA in injections was evaluated from external calibration curve.

HPLC procedure

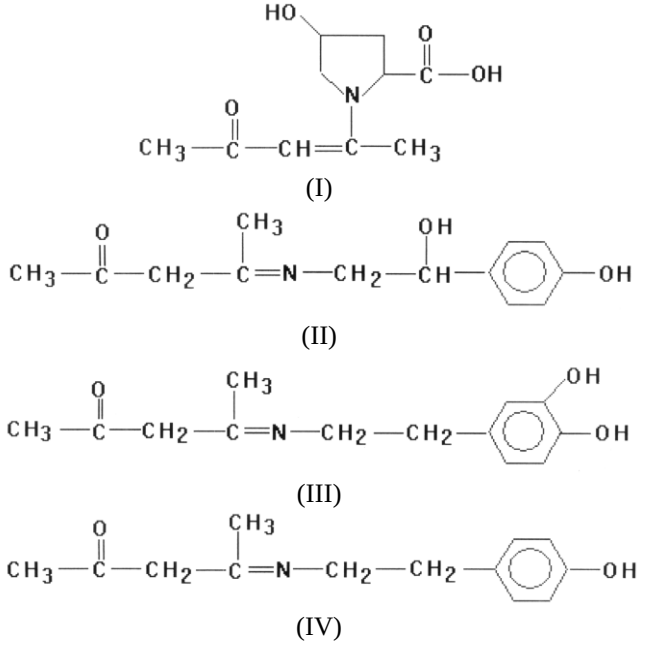
Aqueous solution (1ml) containing dopamine (12.8-189.0µg), HP (14.4 -72.0µg), OP (15.0-76.0µg) and TY (40-200µg) was transferred to 5m1 volumetric flask and was treated as (I). The volume was adjusted to the mark with methanol and solution l0µl was injected on the column Phenomenex C-18 (150 x 4.6mm id). The elution was carried out with a ternary mixture of methanol: acetonitrile: water (45: 6: 49 v/v/v) with a flow rate of 1.2mL mm^{-1} and UV detection was at 320nm.

HPLC analysis of dopamine in pharmaceutical preparations

Solution (1mL) from dopamine injection (5mL) (Abbot Lab. (PVT), Karachi, Pak) and dupont (Knoll Pharmaceuticals Ltd, Korangi, Karachi, Pak.) were diluted to 5mL. The solution (0.lmL) was taken and volume was adjusted to 1mL. The analytical procedure (III) was then followed and the amount of dopamine in injection was evaluated from external calibration curve.

RESULTS AND DISCUSSION

The reagent AA reacts with amino group containing compounds to form highly stable Schiff bases. The reactions of AA were examined towards DA, HP, OP and TY to form derivatives in molar ratio 1:1 (fig. 1). The reactions were initially monitored spectrophotometricaily to optimize the reaction conditions for DP. The conditions which gave maximum responses were considered as optimal. Variation in pH was examined within the range 1-10 at unit interval. The maximum absorbance was observed using carbonate buffer pH 9 (fig. 2). Heating time at 75ºC was varied from 5mm to 30mm. The reaction of DA with AA required 20mm warming time. The amount of AA (0.4%v/v in water) added was varied between 0.5 to 3.0 ml at an interval of 0.5 ml. A similar response was observed after the addition of 1ml and 1.5m1 was considered as optimal.



(I) (II) (III) (IV)

Fig. 1: Structural formula (i) Hydroxyproline-AA, (ii) octopamine-AA (iii) dopamine-AA (iv) Tyramine-AA.

At optimized conditions DA with AA indicated molar absorptivity of 6200 L $mole^{-1}$ cm^{-1} at 318 nm with calibration range of 4.5-45.5µgmL^{-1} with coefficient of determination (r^2) = 0.9993 and y= 0.0293x. The spectryphotometric data of reactions of HP, OP, and TY with AA is summarized in (table 1). All the derivatives were observed highly stable and did not show any change in absorbance upto 24h. 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 reported values with C.V 1.4-2.6%. The presence of hydrochloric acid, sodium acetate, citric acid and sodium citrate in the pharmaceutical preparation did not affect the determination.

Keeping in view the spectrophotometric data an attempt was made to develop reversed phase HPLC method for the determination of DA by UV detection. Each of the derivatives of DP, HP, OP and TY with AA eluted from Phenomenex C-18 (150 x 4.6mm id) column as sharp peaks

with binary mixtures of methanol- water. The detection UV was at 320 nm. However complete separation between DP, HP, OP and TY was obtained when eluted isocratically with a ternary mixture of methanol: acetonitrile: water (45: 6: 49v/v/v) using a flow rate of 1.2 $mLmin^{-1}$. The resolution factor (Rs) between two adjacent peaks was >1.5. The derivatizing reagent AA did not absorb at 320nm, therefore the response of AA was not visible in chromatograph (fig. 3).

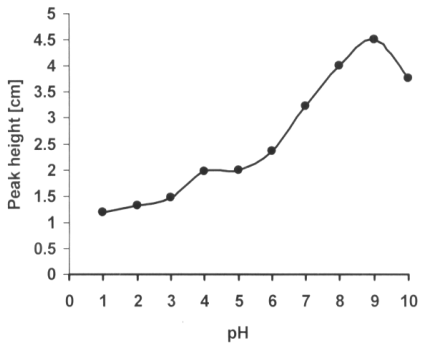


Fig. 2: Effect of pH on the absorbance of AA-DA derivative.

Table 1: Quantitative spectrophotometric data of AA derivatives in methanol-water

Name of Derivative	Wavelength nm	Molar Absorptivity $Lmole^{-1}$ cm^{-1}
Dopamine-AA	318	6200
Hydroxyproline-AA	322	5460
Octopamine	316	11828
Tyramine	318	9810

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 linear calibration curves for DA, HP, OP and TY were obtained by recording average peak height (n=3) versus concentration with 2.5-37.8μgml^{-1}, 2.88-14.4 μgml^{-1}, 3.0-15.2 μgml^{-1} and 8.0-40.0 μgml^{-1} respectively, with detection limits of 7.5ng, 8.0ng, 2.9ng and 3.0ng $injection^{-1}$ (l0 µl) respectively. The regression equation for DA was calculated as y= 0.3009x with coefficient of determination r^2 =0.9995. The reproducibility for the determination of DP was checked with 5.12 μgml^{-1} in terms of peak height and retention time and C.V were obtained 3.4% and 2.4 % (n=6) respectively. The test solutions of DA were analyzed by using AA as derivatizing reagents and relative % errors were observed within ± 0.6- 2.8% respectively.

The effect of different amino acids on the determination of DA was examined. The amino acids glycine, phenylanaline, tryptophan, serine, leucine, valine, arginine, tyrosine, L-histadine, L-spargine, arginine, L-lysine and isolucine when added at the same concentration as that of DA (5.12 μgml^{-1}) did not affect the determination of DA. Ascorbic acid and uric acid when added at the same concentration as DA also did not interfere.

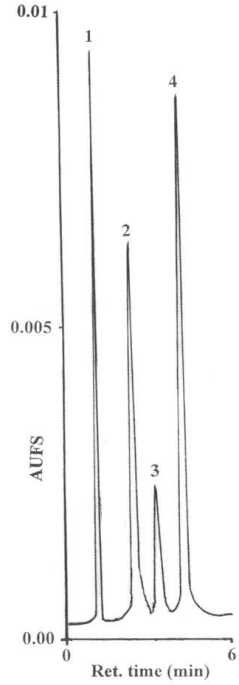


Fig. 3: HPLC separation of derivatives of AA, 1 = 4-hydroxyproline, 2=octopamine, 3=dopamine, 4=tyramine. Column Phenomenex C-18, Sµm (150x4.6mm id), eluant methanol: Acetonitrile: water (45: 6: 49v/v/v), flow rate $1.2mlmin^{-1}$, UV detection 320nm.

Two DA injections (Dopamine hydrochloride and Intropin Dupont) were analyzed for contents of DA. An aliquot of sample after appropriate dilution was subjected to derivatization procedure and amount of DA was evaluated from external calibration. The results (table 2) indicated C.V 0.7-1.3%

CONCLUSION

Simple analytical procedures using spectrophotometer and HPLC connected with UV detection have been described for the determination of DA in the presence of HP, OP and TY using AA as derivatizing reagent. The detection limits

Table 2: Analysis of pharmaceutical preparations by HPLC using AA as derivatizing reagent

Name of preparations	Compounds present	Amount reported mg mL^{-1}	Amount found mg mL^{-1} (CV %)	% RD
Dopamine HCL	Dopamine HC1, Citric acid, Sodium citrate buffer	40	39.2 (0.7)	2.0
Intropin Dupont	Dopamine HCl	40 (1.3)	38.5	3.75

obtained were 1.9 to 8ng injection^{-1}. The method could be used for the determination of DA from pharmaceutical preparations.

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