Asian Journal of Chemistry; Vol. 23, No. 12 (2011), 5419-5421

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Indirect Determination of Catecholamines in Pharmaceutical Preparations by Flame Atomic Absorption Spectrophotometry

MUHAMMAD ASLAM MIRZA[1,*], MUHAMMAD AZIZ CHOUDHARY[1], ABDUL JABBAR KANDHRO[2], MUHAMMAD YAR KHUHAWAR[2], RAFEE ARAIN[2] and TAZ MUHAMMAD JHANGIR[2]

[1]Mirpur University of Science and Technology, Mirpur, Pakistan
[2]Institute of Advanced Research Studies in Chemical Sciences, University of Sindh, Jamshoro, Pakistan

*Corresponding author: Tel: +92 58 27961100; E-mail: ma_mirza64@yahoo.com

(*Received*: 23 December 2010; *Accepted*: 23 August 2011) AJC-10297

Flame atomic absorption spectrophotometer was used for the determination of catecholamines; dopamine, adrenaline and noradrenaline based on the precipitation of catecholamines as copper(II) complex and determination of remaining copper(II) in solution by flame atomic absorption spectrophotometer. A decrease in concentration of copper(II) at pH 7 was proportional to catecholamines concentration. Linear calibration curves for dopamine, adrenaline and noradrenaline were obtained within 0.2-2.5 µg/mL with coefficient of determination (R^2) within 0.9966-0.9991. A number of pharmaceutical additives tested did not affect the determination. Dopamine, adrenaline and noradrenaline in pharmaceutical preparations with relative standard deviation within 0.13-1.6 % with relative deviation from labeled values within 0.2-4.0 %.

Key Words: Catecholamines, Copper complex, Adrenaline, Noradrenaline, Dopamine, Flame atomic absorption spectrophotometer.

INTRODUCTION

Catecholamines show strong pharmacological action in numerous physiopathological processes. Among these dopamine, adrenaline and noradrenaline are common drugs. They play main role as neurotransmitters and also on cardioneopathus in association with free radical mechanism[1]. The impairment of their metabolism is incriminated in Schizophrenia and Perkins disease[2,3].

Flame atomic absorption spectrophotometer (FAAS) is extensively used for the determination of metal ions, because of high sensitivity, low limits of detection, low running cost and ease of the operation of the equipment. Flame atomic absorption spectrophotometer is also used for the analysis of organic compounds, pharmaceutical preparations and biologically active compounds[4-10]. The methods are based on the determination of complexed metal ions or decrease in the concentration of metal ions due to complexation. Catecholamines can bind to the active sites of copper(II) in the enzymes[11]. The catecholamines indicate a vigorous turn over and changed distribution in the experimental copper deficient animals[12,13]. The complex formation between copper(II) ions and dopamine, adrenaline and noradrenaline in aqueous solution has been reported[14-16].

The work examines the reaction of dopamine, adrenaline and noradrenaline with copper for quantitative precipitation as copper(II) chelate and measurement of a decrease in the concentration of copper(II) in solution by flame atomic absorption spectrophotometer, corresponding to dopamine, adrenaline or noradrenaline.

EXPERIMENTAL

Dopamine (DA), adrenaline (AD), noradrenaline (NA) and methanol (E-Merck, Darmstadt, Germany) were used. Hydrochloride acid (37 %), potassium chloride, acetic acid, sodium acetate, ammonium chloride and ammonia (35 %) were from E-Merck, Germany.

The stock solution containing 1 mg/mL of dopamine, adrenaline and noradrenaline were prepared by dissolving appropriate amount in methanol: water (1:1 v/v) and volume was made up to 25 mL. The stock solutions were kept at 4 ºC and diluted as required with methanol: water (1:1 v/v) on the same day.

The buffer solutions in the pH range 1-10 at unit interval were prepared from hydrochloric acid (0.1 M) and potassium chloride (0.1 M) (pH 1-2), acetic acid (0.1 M) and sodium acetate (0.1 M) (pH 3-6), ammonium acetate (0.1 M) and acetic acid (0.1 M) (pH 7), boric acid (0.1 M) and sodium tetra borate (0.1 M) (pH 8), sodium bicarbonate (0.1 M) and sodium carbonate (0.1 M) (pH 9), and ammonium chloride (0.1 M) and ammonia (0.1 M) (pH 10).

The pH measurement was made with an Orion 420 A pH meter (Orion Research Inc, Boslon, USA) with combined glass electrode and reference internal electrode.

Flame atomic absorption studies were carried out using Varian Spectr AA-20 spectrophotometer with standard burner head at the conditions recommended by the manufacturer. The analysis was carried out in quadruplet (n = 4) with delay time 3 s and integration time 3 s.

Analytical procedure

Determination of dopamine, adrenaline and noradrenaline: Aqueous solution 2 mL each of dopamine, adrenaline and noradrenaline (0.2- 12.5 µg/mL) were taken ina volumetric flask (10 mL) separately. Ammonium acetate buffer pH 7 (1 mL) and copper(II) sulphate solution (20 µg/mL) were added in each flask and volume was adjusted up to the mark with deionized water.

The contents of each flask were mixed well and kept at room temperature for 10 min. The contents were centrifuged at 3000 rpm for 10 min and filtered with Watmann filter paper No. 41. The reagent blank was prepared by following the same procedure, but addition of dopamine, adrenaline and noradrenaline was omitted. The unreacted copper(II) was determined by flame atomic absorption spectrophotometer at 324.8 nm with slit width 0.5 nm using air acetylene flame. The calibration curves were constructed by plotting concentration *versus* absorbance values.

Determination of dopamine, adrenaline and noradrenaline from pharmaceutical preparations: Dopamine injection (5 mL, 40 mg/mL) (Abot Lab Pvt. Karachi, Pakistan), adrenaline injection (1 mL, 1.0 mg/mL) (Venous Pharma, Pakistan & Elite Pharma, Pakistan) and noradrenaline compound (E Merck, Germany) were appropriately diluted to obtain concentration within calibration range (0.25-5 µg/mL). The solution 1 mL was transferred to 10 mL volumetric flask and analytical procedure was followed. The quantitation was made from external calibration curve.

RESULTS AND DISCUSSION

Dopamine, adrenaline and noradrenaline reacted with excess of copper(II) to form the complexes and precipitated out from aqueous solution. The copper(II) solution after filtration was analyzed by flame atomic absorption spectrophotometer. The effect of pH and temperature on the complex formation was examined using analytical procedure. The effect of pH was examined between 1-10 at unit operation. The complexation was observed between pH 3-9 but somewhat better response was observed at pH 7 and was selected (Fig. 1). Effect of temperature was varied between 5-55 ºC in water bath at an interval of 10 ºC. Maximum complexation and precipitation of catecholamines with Cu(II) occurred at 25 ºC and was selected (Fig. 2).

Linear calibration curves were constructed by recording average absorbance of copper(II) solution (n = 4) against the concentration of dopamine, adrenaline and noradrenaline and were obtained within 0.25 - 2.5 µg/mL of dopamine, adrenaline and noradrenaline (Fig. 3). The coefficients of determination (R^2) were obtained 0.9991, 0.9966 and 0.9986 for dopamine, adrenaline and noradrenaline with linear regression equations y = - 0.0269x + 0.0982, y = - 0.304x + 0.1163 and y = -0.0298 x + 0.1303 respectively (Table-1). The analysis was repeatable with 1 mg/mL for dopamine, adrenaline and noradrenaline (n = 4) with RSD within 3 %. The effect of additives such as glucose, magnesium stearate, gum acacia, talcum, methylparabin, starch and lactose on the determination of dopamine, adrenaline and noradrenaline was also examined. The analytes and additives were mixed in the ratio 1:10 w/w. No significant effect of additives was observed on the determination of dopamine, adrenaline and noradrenaline with relative error within ± 0.7 - 2.3 %.

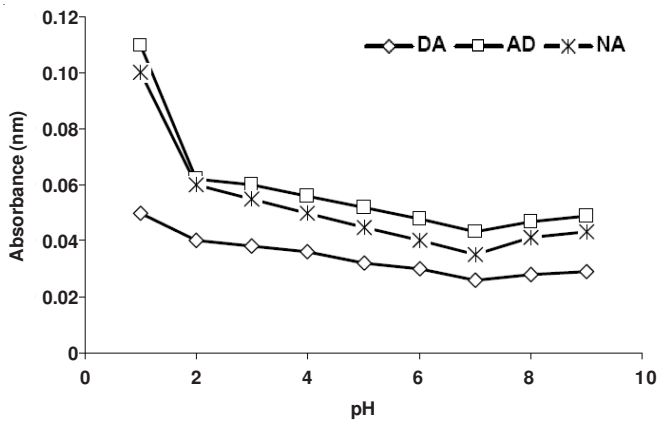

Fig. 1. Effect of pH on complexation of dopamine (DA), adrenaline (AD) and noradrenaline (NA) with Cu(II) (n = 4)

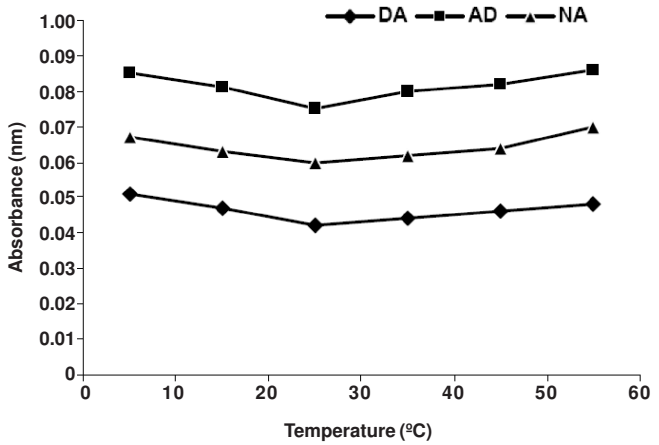

Fig. 2. Effect of temperature on complexation of dopamine (DA), adrenaline (AD) and noradrenaline (NA) with Cu(II) (n = 4)

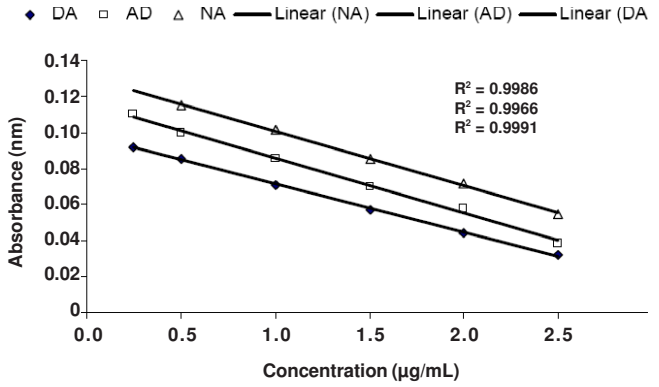

Fig. 3. Calibration curves of dopamine (DA), adrenaline (AD) and noradrenaline (NA) (n = 4)

TABLE-1
QUANTITATIVE AAS RANGES FOR THE DOPAMINE, ADRENALINE AND NORADRENALINE AFTER COMPLEXATION WITH Cu(II)

Name of compounds	Calibration range (μg/mL)	LOD (μg/mL)	Coefficient of determination (R^2)	Regression equation
Dopamine	0.25-2.50	0.25	0.9991	Y = – 0.0269x + 0.0982
Adrenaline	0.25-2.50	0.25	0.9966	Y = – 0.0304x + 0.1163
Noradrenaline	0.5-2.50	0.5	0.9986	Y = – 0.0298x + 0.1303

TABLE-2
ANALYSIS OF DOPAMINE, ADRENALINE AND NORADRENALINE IN PHARMACEUTICAL PREPARATIONS

Name of manufacturing company	Compounds present	Amount reported (μg/mL)	Amount found (μg/mL) (C.V. %)	(%) RSD
Dopamine HCl (Abbot Lab., Karachi, Pak.)	Dopamine HCl, citric acid, sodium citrate buffer	40	38.40 (0.9)	4.0
Dopamine HCl (Abbot Lab., Lahore, Pak.)	Dopamine HCl, citric acid, sodium citrate buffer	40	39.18 (0.2)	2.0
Dopamine HCl (Merck)	Dopamine HCl	0.5 (Taken)	0.51 (0.13)	1.4
Adrenaline (Elite Pharma Pak.)	Adrenaline	1.0	1.02 (1.3)	2.4
Adrenaline (Venus Pharma Pak.)	Adrenaline	1.0	0.99 (1.6)	0.6
Adrenaline (Merck)	Adrenaline	0.5 (Taken)	0.48 (0.15)	0.2
Noradrenaline (Merck)	Noradrenaline	0.5 (Taken)	0.52 (0.18)	0.2
Noradrenaline (Merck)	Noradrenaline	1.0 (Taken)	0.97 (0.65)	3.0

For the application of the developed method, dopamine and adrenaline injections purchased from local market (Hyderabad, Pakistan) were analyzed. Noradrenaline was not available in dosage form hence test solution prepared from standard noradrenaline solution within the calibration range was analyzed to examine the recovery of noradrenaline.

The results are summarized in Tables 1 and 2 and indicated RSD within 0.1-1.6 % and standard deviation from the labeled volumes within 0.2-4.0 %.

Conclusion

Simple analytical procedure using flame atomic absorption spectrophotometer has been developed for the determination of dopamine, adrenaline and noradrenaline within the liner range 0.25-2.5 μg/mL by complexation with copper. The complexation and precipitation with Cu(II) was rapid and repeatable for the analysis of dopamine, adrenaline and noradrenaline from pharmaceutical preparations.

REFERENCES

1. P.K. Singal, N. Kapur, K.S. Dhillon, R.E. Beamish and N.S. Dhalla, *Can. J. Physiol. Pharmacol.*, **60**, 1390 (1982).
2. Y.C. Clement-Cormier, J.W. Kebabian, G.L. Petzold and P. Greengard, *Proc. Natl. Acad. Sci. USA*, **71**, 1113 (1974).
3. M.E. Pyeyo, W. Gonzalez, E. Pussard and J.F. Amal, *Diabetologia*, **37**, 879 (1994).
4. A.A. Salem and M. Omar, *Turk J. Chem.*, **27**, 383 (2003).
5. G.H. Ragab and A.S. Amin, *Spectrochim. Acta A*, **60**, 2831 (2004).
6. J.M. Catatayud and J.V.G.Mateo, *Analyst,* **116**, 327 (1991).
7. H. Salem, W. Khater and L. Fada, *Am. J. Pharmacol. Toxicol.*, **2**, 65 (2007).
8. G. Alpdoðan and S. Sungur, *Anal. Lett.*, **32**, 2799 (1999).
9. M.A. El-Reis, F.M.A. Attia and I.M.M. Kenawy, **23**, 249 (2000).
10. M.Y. Khuhawar, T.M. Jahangir and F.M. Rind, *J. Chem. Soc. Pak.*, **23**, 226 (2001).
11. E. Walaas, O. Walaas and S. Haavaldsen, *Arch. Biochem. Biophys.*, **100**, 97 (1963).
12. J.R. Prohaska, W.R. Bailey, A.M. Gross and J.J. Korte, *J. Nutr. Biochem.*, **1**, 149 (1990).
13. M. Fields, C.G. Lewis and M.D. Lure, *Metabolism*, **40**, 1 (1991).
14. A.C. Andrews, T.D. Lyons and T.D. O'Brien, *J. Chem. Soc.*, 1776 (1776).
15. R.F. Jemeson and W.S.F. Neilie, *J. Chem.*, **27**, 2391 (1965).
16. R.F. Jemeson and W.S.F. Neilie, *J. Inorg. Nucl. Chem.*, **28**, 2667 (1966).