Capillary-Gas Chromatographic Determination of Phenylpropanolamine Using Acetylacetone as Derivatizing Reagent in Pharmaceutical Preparations

[1]KULSOOM ABBASI, [1]MUHAMMAD IQBAL BHANGER
AND [2]MUHAMMAD YAR KHUHAWAR*
[1]*National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan.*
[2]*Dr. M. A. Kazi Institute of Chemistry University of Sindh, Jamshoro, Pakistan.*

(Received 14th January 2008, revised 12th April 2008)

Summary: An analytical procedure has been developed for the gas chromatographic determination of phenylpropanolamine hydrochloride (PPA. HCl) using acetylacetone (AA) as derivatizing reagent. Elution is carried out from the column HP-5 (30m X 0.32 mm i.d) with film thickness 0.25 μm at initial column temperature 70°C for 5 min, followed by heating rate 10°C/ min up to 120°C with total run time 20 min. Injection port and detector temperatures were maintained at 270°C and 300°C. Nitrogen flow rate was 2ml/ min and detection was by FID. The split ratio was 10:1. The linear calibration was obtained with 50 to 750 μg/ ml PPA with detection limit of 15 μg/ ml. Under the condition ephedrine did not form, the derivatives with AA but separated from PPA derivatives. The linear calibration curve for ephedrine was obtained with 225-903 μg/ ml with detection limit of 103 μg/ ml.
The method was used for the determination of PPA from Sinutab and Tavegyl -D tablets, and ephedrine from Ephedrine tablets. The coefficient of variation for the analysis of pharmaceutical preparation was obtained within 0.78 % to 0.89 %.

Introduction

Phenylpropanolamine hydrochloride (PPA. HCl) is a sympathomimetic agent with vasoconstrictor and decongestant effects on inflamed mucous membranes. It is also reported as an appetite suppressant. It is used in over-the counter (OTC) and prescription medications for cough and cold. Recently, considerable interest in PPA.HCl has arisen due to the serious side effects accompanying its use including hemorrhagic stroke, arrhythmias and hypertension [1-5]. This caused Federal Drug Authority (FDA) of United States to ask OTC manufactures to reformulate products containing PPA to remove PPA from the market and issue public advisory warning about the risks linked to PPA.HCl [6-8].

A number of analytical methods have been reported for the determination of PPA mostly based on spectrophotometry [9], room temperature phosphorescence [10], fluoroimmunoassay [11], radioenzymic assay [12], Raman spectroscopy [13], capillary electrophoresis [14,15], thin layer [16], gas chromatography (GC) [17-23] and liquid chromatography (LC) [24-33]. The procedures based on GC and LC are more widely reported. The GC methods are based on precolumn derivatization with N-methyl-N-trimethylsilyl-trifluoroacetamide [21], N-methyl-bis-(heptafluorobutyramide) [34], heptafluorobutyric anhydride [35] and cyclohexanone [35]. However some column damage has been reported using acetylation reagents. The present work examines acetylacetone (AA) for the GC determination of PPA in pharmaceutical preparations.

Results and Discussion

Phenylpropanolamine derivatives formed with AA (Fig. 1) absorbed at 316 nm with molar absorbtivity of 9134 $mol^{-1}cm^{-1}$ and supported the formation of effective derivatives. Change in absorbance was not observed up to 24 hours. After extraction of amine in chloroform from alkaline solution, the derivatization was carried out in chloroform-methanolic media, in the presence of acetic acid. For better sensitivity GC was examined for the determination of PPA. The elution of the AA-PPA derivatives formed was investigated from the capillary column HP-5 (30m X 0.32 mm i.d). The derivative eluted at the column temperature 120°C with nitrogen flow rate of 2 ml/ min as single peak and separated completely from the derivatizing reagent. The effect of pH, the concentration of derivatizing reagent and heating time on the derivatization were examined. Each time a constant

*To whom all correspondence should be addressed.

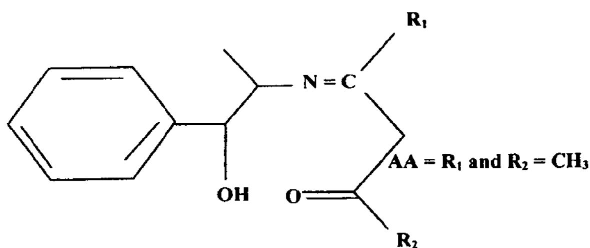

Fig. 1: Structural diagram PPA derivative.

volume 1μl was injected, average peak area (n =3) was noted. The condition, which gave maximum response, was considered as optimum. The effect of pH was examined within 1-10, a similar response was obtained within pH 1-3.5. A significant decrease in the response was observed above the pH 3.5. It was therefore addition of acetic acid which covered the pH range satisfactorily.

The amount of derivatizing reagent added was varied from 0.5-2.5 ml (1 % v/ v) at an interval of (0.5 ml) and addition of 1ml was considered as optimum. Heating time at 75°C was varied from 5-25 min at an interval of five min and a similar response was obtained after heating for 10 min and was selected.

Linear calibration curves were obtained with 50-750μg/ ml PPA with coefficient of determination r^2 = 0.9995. The detection limit measured as three times the background noise was obtained 15μg/ ml corresponding to 15 *ng*/ injection (1μl) and 1.5 *ng* reaching to the detector with split ratio of 10:1.

The effects of additives present in the pharmaceutical preparations were examined for the possible interfering effect on the determination of PPA. The additive was added at least twice the concentration of PPA. Magnesium stearate, gum acacia, methyl paraben, lactose, starch, glucose and talcum did not affect the determination with relative deviation within 3 %.

Clemastine hydrogen fumarate and phenyltoloxamine citrate are commonly present in pharmaceutical preparations together with PPA. Their effects on the determination of PPA were examined. PPA is selectively extracted from alkaline medium in chloroform and clemastine hydrogen fumarate and phenyltoloxamine citrate remained in aqueous-methanol solution and did not affect the GC determination of PPA.

The reproducibility of the determination of PPA was examined in terms of average peak height, average retention time (n=5) and relative standard deviation (RSD). RSD were obtained 0.89 % and 0.81 % respectively. PPA in pharmaceutical preparation is present separately, but its possible separation from ephedrine was examined. Ephedrine was added together with PPA and analytical procedure was followed. Ephedrine contains secondary amino group and did not react with AA to form the derivative. However when injected on the column HP-5 (30 m × 0.32 mm id) at a column temperature 70° for 5 min, followed by heating rate 10°C/ min up to 120°C with total run time 20 min. with nitrogen flow rate 2 ml/ min, a reasonable peak shape was obtained for ephedrine and separated completely from PPA-AA derivative (Fig. 2). Linear calibration range was within 225-903 μg/ ml with coefficient of determination (r^2) 0.9988 and Y= 12.34x. The detection limit was obtained 103 μg/ ml. The pharmaceutical preparations were analyzed for the contents of PPA and ephedrine. The results are summarized in Table-1 and indicate correlation with labeled values. The results indicate RSD within 0.81 - 0.90 indicating recovery from the pharmaceutical preparations was within 94-98.6 %.

Table-1: Analysis of phenylpropanolamine, and ephedrine from pharmaceutical preparations.

S. No	Name of Compound	Name of Drug	Amount Labeled mg/ tablet	Amount Found mg/ tablet (RSD %)	% Recovery
	PPA	Tavegyl-D	75	73 (0.89%)	97.3%
		Sinutab	25	24.68 (0.81%)	98.6%
3	Ephedrine	Ephedrine	30	28.5 (0.9%)	94%

Experimental

Acetylacetone (Fluka), phenylpropanolamine hydrochloride (norephedrine hydrochloride) (Sandoz, Pak.), ephiderine hydrochloride [(1 methylaminoethyl) benzylalcohol hydrochloride] (Merck), methanol (E. Merck), chloroform (E. Merck) and sodium hydroxide (Fluka) were used. Spectrophotometric studies were carried out using a double beam Hitachi 220 spectrophotometer. pH measurements were made with Orion 420 A pH meter with glass electrode and internal reference electrode (Orion Research Inc. Boston, USA).

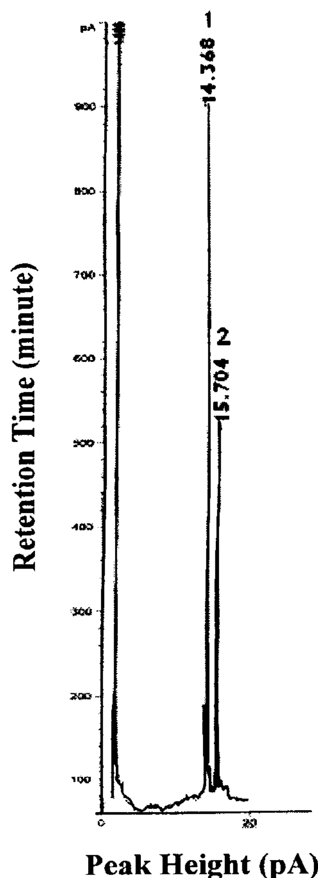

Fig. 2: GC separation of (1) PPA-AA (2) Ephedrine. Condition as experimental.

The gas chromatographic studies were carried out on Agilent model 6890 N Net work GC system gas chromatograph (Agilent Technologies, Inc. USA) connected with flame ionization detection (FID), hydrogen generator Parker Balson model H2-90, Analytical gas system (Parker Hannifin Haverhill MA, USA) and pure nitrogen (British Oxygen Company, Karachi). The gas chromatograph was controlled by the computer with Chemstation soft ware (Agilent Technologies). HP 1300 laser jet printer was used through out the study. Capillary GC column HP-5, (30mx 0.32 mm i.d) with film thickness 0.25 μm (J &W Scientific GC column, USA) was used throughout the study.

Analytical Procedure

To the solution of phenylpropanolamine hydrochloride (0.2-1ml) containing (50-750 μg) ephedrine hydrochloride, (225-903 μg) sodium hydroxide (0.5ml, 0.2 %w/v) in water and chloroform (2 ml) were added. The contents were mixed well and layer was allowed to separate. Exactly (1ml) of chloroform layer was transferred to screw cap vial acetylacetone (1ml, 1 % v/v) in methanol and acetic acid (0.1ml) and were added. The contents were heated at 75°C for 10 min and the residue was dissolved in methanol (0.5ml). The solution (1μl) was injected on the column HP-5 (30m x 0.32 mm i.d) with film thickness 0.25 μm at column temperature 70°C for 5 min, followed by heating rate 10°C/ min up to 120°C with total run time 20 min. Injection port and detector temperatures were maintained at 270° and 300°C, nitrogen flow rate was 2 ml/ min with split ratio 10:1. The detection was by FID.

Analysis of Phenylpropanolamine in Pharmaceutical Preparation

Ten tablets each Tavegyl-D (Sandoz Pak. Ltd., Karachi) and Sinutab (Parker- Davis and Co, Pak. Ltd, Karachi) were well ground. Tavegyl – D 0.512g and Sinutab 0.475g were dissolved in water separately. The solution was filtered and volume was adjusted to 10 ml. The solution (0.5ml) for Tavegyl-D and (1ml) for Sinutab were taken and processed as above. The amounts of drugs from pharmaceutical preparations were calculated using external calibration curve.

Determination of Ephedrine in Pharmaceutical Preparation

Five ephedrine tablets (Karachi Chemicals Ltd, Karachi) were powdered and amount corresponding to one tablet (0.1612 g) was weighed and dissolved in water. The solution was filtered and volume was adjusted to 10 ml. Solution (0.2 ml) was taken and analytical procedure was followed but the addition of derivatizing reagent AA was omitted. The solution (1 μl) was injected on the capillary column HP-5 (30m × 0.32 mm i.d) and eluted according to analytical procedure.

Conclusion

Capillary GC procedure has been developed for the determination of phenylpropanolamine and ephedrine after precolumn derivatization of PPA with AA. The detection limits have been achieved at sub ng level reading to FID detection. The method works successfully when used for the determination of PPA from pharmaceutical preparations with recovery of

94-98 %. Ephedrine if present together with PPA could also be separated and determined.

References

1. S. M. Muller, J. Muller and S. M. Asdell. Stroke, *J. Cerebral Circulation.*, **15**, 119 (1984).
2. C. R. Lake, G Zaloga, J. Bray, D. Rosenberg and B. A. Chemow, *J. Med.*, **86**, 427 (1989).
3. C. R. Lake, S. Gallant, E. Masson and P. Miller. *Am. J. Med.*, **89**, 195 (1990).
4. R. Oosterbaan and M. J. Burns, *J. Emer. Med.*, **18**, 55 (2000).
5. W. N. Kernan, C. M. Viscoli, L. M. Brass, J. P. Broderick, T. Brott, E. Feldmann, L. W. Morgenstern and J. L. Wilterdink, *New Engl, J. Med.*, **343**, 1826 (2000).
6. S. W. Toennes, S. Harder, M. Schramm, C. Niess and G. F. Kauert, *J. Clin. Pharmacol.*, **56**, 125 (2003).
7. S. A. Shama and A. S. Amin, *Spectrochim Acta Part A. Mol. Biomol. Spectros.*, **60**, 1769 (2004).
8. C. F. Ferreyra and C. S. Ortiz, *J. Pharm. Biomed. Anal.*, **29**, 811 (2002).
9. L. L. Shankle, *J. Pharm. Sci.*, **67**, 1635 (1978).
10. W. J. Long, R. C. Norin and S. Y. Su, *J. Anal. Chem.*, **7**, 2873 (1985).
11. S. A. Eremin, A. V. Simirnov, G. Gallacher, D. S. Smith and D. L. Colbert, *Analyst.*, **118**, 1325 (1993).
12. A. A. Reid, P. J. Fleming and C. R. Lake, *Anal. Biochem.*, **165**, 275 (1987).
13. T. H. King, C. K. Mann and T. J. Vickers, *J. Pharm. Sci.*, **74**, 443 (1985)
14. C. E. Lin, I. J. Fang, Y. J. Deng, W. S. Liao, H. T. Cheng and W. P. Huang, *J. Chromatogr. A.*, **1051**, 85 (2004).
15. Y. T. Iwata, A. Garcia, T. Kanamori, H. Inoue, T. Kishi and I. S. Lurie, *J. Electrophoresis.*, **23**, 1328 (2002).
16. A. Wu. D. D. Bretl, M. L. Pearson, G. S. Holffe and M. L. Miller, *Clin. Chem.*, **32**, 407 (1986).
17. A. Dasgupta and A. P. Hart, *J. Forensic Sci.*, **42**, 106 (1997).
18. J. Guerra, D. Carreras, C. Rodriguez, A. F. Rodriguez and R. J. Cortes, *J. Chromatogr B Biomed.*, **687**, 183 (1996).
19. P. Van Eenoo, F. T. Delbeke, K. Roels and P. DeBacker, *J. Chromatogr. B.*, **760**, 255 (2001).
20. M. E. Spyridaki, C. J. Tsitsimpikou, P. A. Siskos and C. G. Georgakopoulos, *J. Chromatogr. B. Biomed. Sci. Appl.*, **758**, 311 (2001).
21. G. Forsdahl and G. Gmeiner, *J. Chromatogr B.*, **811**, 201 (2004).
22. Y. L. Tseng, H. R. Hsu, F. H. Kuo, M. H. Shieh and C. F. Chang, *J. Anal. Toxicol.*, **27**, 359 (2003).
23. B. M. El-Haj, A. M. Al-Amri, M. H. Hassan, H. S. Ali and R. K. Kadam, *J. Forensic Sci. Int.*, **135**, 16 (2003).
24. M. J. Bogusz, M. Kala, R. D. Maider, *J. Anal. Toxicol.*, **21**, 59 (1997).
25. R. Herraz-Hernandez, P. Campins-Falcs and A. Sevillano-Cabeza, *J. Chromatogr. Sci.*, **35**, 169 (1997).
26. M. S. Fuh and K. J. Lu, *Talanta.*, **48**, 415 (1999)
27. M. Gil-Agusti, J. R. Torres-Lapasiso, M. C. Garcia-Al-varez-Cogue and J. Esteve-Romoero, *J. Chromatogr A.*, **866**, 35 (2000).
28. M. C. Roman, *J. A.O.A.C. Int.*, **87**, 15 (2004).
29. A. Kaddoumi, T. Mori, N. Toyomi, N. Mihoko, M. Wada and K. Nakashima, *J. Pharma. Biomed. Anal.*, **34**, 643 (2004).
30. K. Nakashima and S. Kenichiro, *J. Biomed. Chromatogr.*, **16**, 463 (2002).
31. S. J. Sheu and M. H. Huang, *J. Chromatogr.*, **54**, 117 (2001).
32. A. Kaddoumi, M. N. Nakashima and K. Nakashima, *J. Chromatogr. B.*, **763**, 79 (2001).
33. M. Gil-Agusti, L. Monferrar-Pons, M. C. Garcia-Alvarez-Coque and J. Esteve-Romero, *Talanta.*, **54**, 621 (2001).
34. S. W. Toennes, M. Schramm, G. F Kauert, Problemstudie mit Kath-Bioanalytik und forenshische Bewertung, in: Pragst F, R. Aderjan (Eds) Proceedings of the 12th GTFCH Symposium in Mosbach (Baden), Verlag Dr. Dieter Helm, Heppenheim, (2001) p. 162.
35. F. Sporkert, F. Pragst, R. Bachus, F. Masuhr and L. Harms, *J. Forensic Sci. Int.*, **133**, 39 (2003).