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Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 998–1001
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Capillary gas chromatographic determination of phenylpropanolamine in pharmaceutical preparation

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Received 18 November 2005; received in revised form 22 December 2005; accepted 3 January 2006
Available online 24 April 2006

Abstract

Analytical procedure has been developed for the gas chromatographic determination of phenylpropanolamine (PPA) using trifluoroacetylacetone (FAA) as derivatizing reagent. Elution is carried out from the column HP-5 (30 m × 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. Injection port temperature was maintained at 270 °C. Nitrogen flow rate was 2 ml/min and detection was by FID.

The linear calibration curve was obtained with 30–150 μg/ml PPA with detection limit of 6.0 μg/ml. The method was used for the determination of PPA from Sinutab and Tavegyl-D tablets. The relative standard deviation (R.S.D.) for the analysis of pharmaceutical preparation was obtained within 0.4–0.9%.
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Keywords: Phenylpropanolamine; Trifluoroacetylacetone; Pharmaceutical

1. Introduction

Phenylpropanolamine hydrochloride (PPAx·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 has increased due to the serious side effects accompanied its use including hemorrhagic stroke, arrhythmias and hypertension [1–5]. This caused Federal Drug Administration (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 [6,7].

A number of analytical methods have been reported for the determination of PPA mostly based on spectrophotometry [8,9], spectrofluorimetery [10], room temperature phosphorescence [11], fluoroimmunoassay [12], radioenzymic assay [13], Raman spectroscopy [14], capillary electrophoresis [15,16], thin layer [17], gas (GC) [18–24] and liquid chromatography (LC) [25–34]. The procedures based on GC and LC have been more frequently reported. The GC methods are based on precolumn derivatization with *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide [22], *N*-methyl-bis-(heptafluorobutyramide) [35,36], heptafluorobutyric anhydride [35] and cyclohexane [35]. However, some column damage problems have been reported using acetylation reagents. The simple β-diketone could react with primary amino compound to form highly stable Schiff base and enhance the sensitivity of FID detection due to increase in carbon chain length. Trifluoroacetylacetone (FAA) has been used for the GC determination of diamino-compounds putresine and cadaverine from biological fluids [37], but the present study examines FAA for the GC determination of PPA.

2. Experimental

2.1. Materials

Trifluoroacetylacetone (Fluka), phenylpropanolamine hydrochloride (norephedrine hydrochloride) (Sandoz Pak.), ephedrine hydrochloride [(1-methylaminoethyl) benzyl alcohol hydrochloride] (Merck), methanol (E. Merck), chloroform

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0731-7085/$ – see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.jpba.2006.01.004

(E. Merck), sodium hydroxide (Fluka), hydrochloric acid (37%), potassium chloride, acetic acid, sodium acetate, ammonium chloride and ammonia (E. Merck) were used. Buffer solutions at unit pH interval were prepared from the following: hydrochloric acid (1.0 M), potassium chloride (1.0 M), acetic acid (1.0 M), sodium acetate (1.0 M), ammonium acetate (1.0 M), ammonia (1.0 M), ammonium chloride (1.0 M), sodium carbonate (saturated) and sodium bicarbonate (1.0).

2.2. Instrumentation

Spectrophotometric studies were carried out using a double beam Hitachi 220 spectrophotometer (Hitachi (PVT) Ltd., Tokyo) with dual 1 cm silica cuvettes. pH measurement were made with Orion 420 A pH meter with glass electrode and internal reference electrode (Orion Research Inc., Boston, USA).

The gas chromatographic studies were carried out on Agilent model 6890 Net work GC system gas chromatograph (Agilent Technologies Inc., USA) coupled 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 (BOC), Karachi). The gas chromatograph was controlled by the computer with Chemstation software (Agilent Technologies). Capillary GC column HP-5 (30 m × 0.32 mm i.d.) with film thickness 0.25 μm (J &W Scientific GC column, USA) was used throughout the study.

2.3. GC–FID method

To the solution of phenylpropanolamine hydrochloride (0.2–1 ml) containing (30–150 μg) was added sodium hydroxide (0.5 ml, 0.2% w/v) in water and chloroform (2 ml). The contents were mixed well and layer was allowed to separate. Exactly 1 ml of chloroform layer was transferred to screw cap vial and was added FAA (1 ml, 1% v/v) in methanol and acetic acid (0.1 ml). The contents were heated at 75 °C for 10 min and the residue was dissolved in methanol (0.5 ml). The solution (1 μl) was injected on the column HP-5 (30 m × 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. FID was used for detection.

2.4. Analysis of phenylpropanolamine from pharmaceutical preparation

Ten tables each Tavegyl-D (Sandoz Pak. Ltd., Karachi) containing 75 mg/tablet and Sinutab (Parker-Davis and Co., Pak. Ltd., Karachi) were well ground. Tavegyl-D 0.512 g and Sinutab 0.475 g in triplicate were dissolved in water separately. The solution was filtered and volume was adjusted to 10 ml. The solution (0.1 ml) for Tavegyl-D and (0.2 ml) for Sinutab were taken and processed as above. The amounts of drug from pharmaceutical preparations were calculated using external calibration curve.

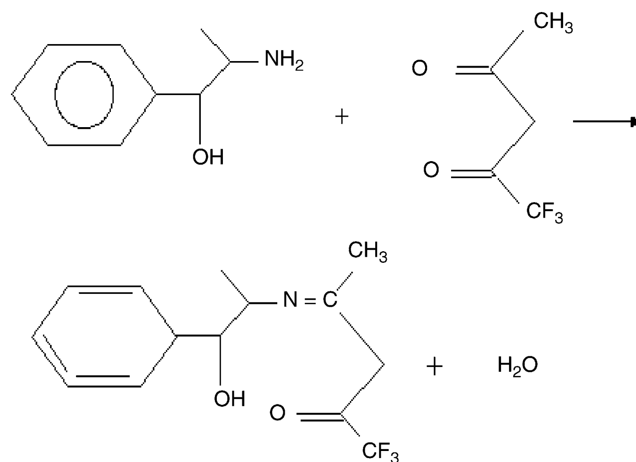

Fig. 1. Schematic reaction of PPA with FAA.

3. Results and discussion

Acetylacetone and FAA were examined for GC determination of PPA, but FAA gave better sensitivity due to the presence of trifluoromethyl group in FAA, which could enhance the thermal stability and volatility of derivative. The derivatizing reagent FAA was, therefore, selected.

PPA derivative formed with FAA (Fig. 1) absorbed at 323 nm with molar absorptivity of 11,211 l/mol cm and supported the formation of derivative. Any change in absorbance up to 24 h was not observed. After extraction of amine in chloroform from alkaline solution, the derivatization was carried out in chloroform-methanolic media, in the presence of acetic acid. The effect of pH, the concentration of derivatizing reagent and heating time on the derivatization was examined. Each time absorbance of the solution was measured against reagent at 323 nm and the condition, which gave maximum response, was considered as optimum. The effect of pH was examined within pH 1–10 and a similar response was obtained within pH 1–3.5 and addition of acetic acid covered the pH range satisfactory (Fig. 2).

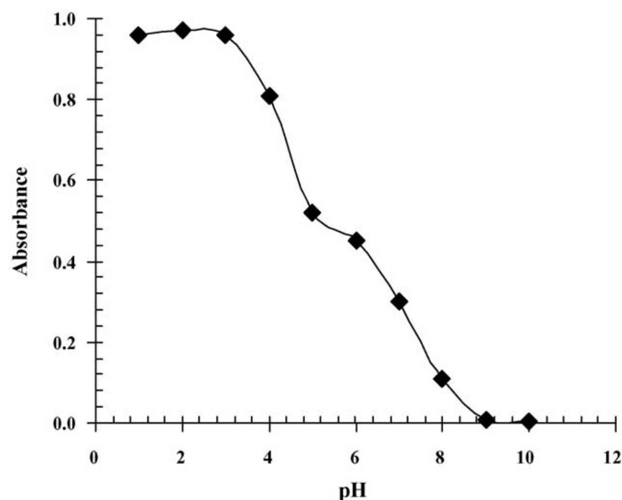

Fig. 2. Effect of pH on derivatization of PPA with trifluoroacetylacetone.

The amount of derivatizing reagent added was varied from 0.5 to 2.5 ml (1% v/v) at an interval of 0.5 ml and a similar response was observed above the addition of 0.5 ml, and addition of 1 ml was considered as optimum. Heating time at 75 °C was varied from 5 to 25 min at an interval of 5 min and a maximum response was obtained by plotting heating of 10 min and was selected.

GC was examined for the determination of PPA. The elution of the FAA–PPA derivative formed was investigated from the capillary column HP-5 (30 m × 0.32 mm i.d.). The derivative eluted at the column temperature 120 °C with nitrogen flow rate of 2 ml/min and single peak was obtained which separated completely from the derivatizing reagent.

Linear calibration curve was obtained by plotting average peak height ($n = 3$) versus concentration with 30–150 μg/ml PPA with coefficient of determination (r^2) 0.997 and $Y = 0.2581x$. The detection limits measured as signal to noise ratio 3:1 was obtained 6 μg/ml, corresponding to 6 ng/injection (1 μl) and 0.6 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 (75 μg/ml). Magnesium stearate, gum acacia, methyl paraben, lactose, starch, glucose and talcum did not affect the determination with relative deviation within 3%.

Clemestine hydrogen fumerate and phenyltoloxamine citrate are commonly present in pharmaceutical preparation together with PPA. Their effects on the determination of PPA were examined. PPA is selectively extracted from alkaline medium in chloroform and clemestine hydrogen fumerate 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 and average retention time ($n = 5$) and relative standard deviation (R.S.D.) were obtained 0.39 and 1.35%, respectively. The test solution of PPA ($n = 4$) were analyzed to cover the whole calibration range and relative percentage error was observed within ±3%

Phenylpropanolamine in pharmaceutical preparation is present separately, but its possible interfering effect from presence of ephedrine was examined. Ephedrine was added together with PPA and analytical procedure was followed. Ephedrine contains secondary amino group and did not react with FAA to form the derivative. However, when injected on the column HP-5 (30 m × 0.32 mm i.d.) it eluted after PPA–FAA derivative. Attempt was, therefore, made to separate ephedrine from PPA–FAA derivative. An optimal separation was obtained when eluted at a column temperature 70 °C for 5 min, followed by heating rate 10 °C/min up to 120 °C with total run time of 20 min with nitrogen flow rate of 2 ml/min. A symmetrical peak was obtained for ephedrine which separated completely from PPA–FAA derivative (Fig. 3). The response of ephedrine did not indicate interference with the determination of PPA. Linear calibration range for ephedrine was within 225–903 μg/ml with coefficient of determination (r^2) 0.9988 and $Y = 12.34x$. The detection limit obtained was 103 μg/ml. The reproducibility for the determination of ephedrine (675 μg/ml) ($n = 3$) was examined in terms of average peak height and retention time and R.S.D. were obtained 0.97 and 1.25%, respectively. The pharmaceutical preparations were analyzed for the contents of PPA. The average results of analyses are summarized in Table 1 and

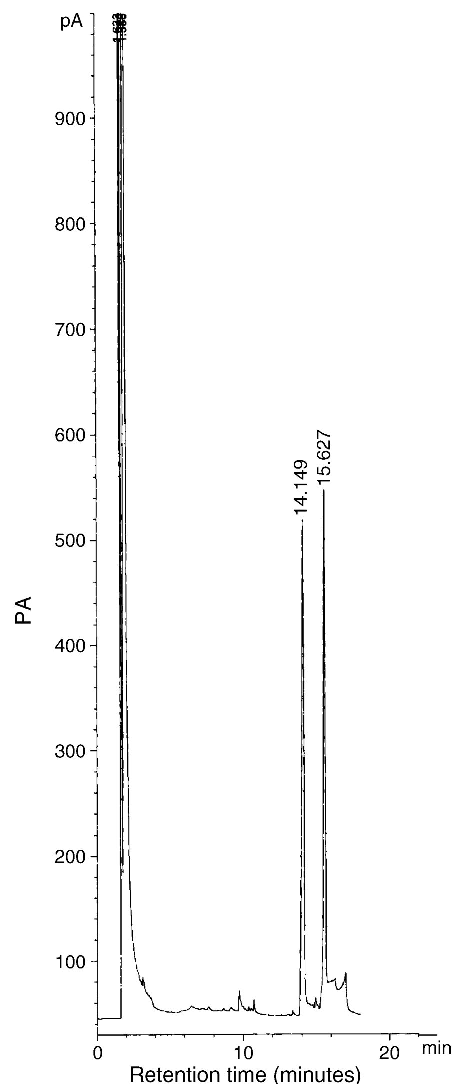

Fig. 3. GC separation of (1) PPA–FAA (2) ephedrine from column HP-5 (30 m × 0.32 mm i.d.) with film thickness 0.25 μm at initial column temperature 70 °C for 5 min, followed by heating rate 10 °C/min up to 120 °C with total run time 20 min. Injection port and detector temperatures were maintained at 270 and 300 °C. Nitrogen flow rate was 2 ml/min and detection was by FID.

Table 1
Analysis of phenylpropanolamine from pharmaceutical preparations

S. no.	Name of compound	Name of drug	Amount present (mg/tablet)	Amount found mg/tablet (R.S.D.%)	%Recovery
1	PPA	Tavegyl-D	75	69.5 (0.4%)	92.7
		Sinutab	25	24.5 (0.4%)	98

indicated close resemblance with reported values and indicated R.S.D. within 0.78% and recovery from the pharmaceutical preparations was within 92.7–98.0%.

4. Conclusion

Capillary GC procedure has been developed for the determination of phenylpropanolamine after precolumn derivatization of PPA with FAA. The detection limit has been achieved as 0.6 ng PPA reaching to FID detection. The method worked for the determination of PPA from pharmaceutical preparations. Ephedrine if present together with PPA could also be separated.

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