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Short communication

HPLC determination of phenylpropanolamine in pharmaceutical preparations using 4-dimethylaminobenzaldehyde as a derivatizing reagent

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Abstract

A method is described for the high performance liquid chromatographic (HPLC) determination of phenylpropanolamine (PPA) based on precolumn derivatization with 4-dimethylaminobenzaldehyde (DAB) and elution from phenomenex C-18 column with methanol–water and detection by spectrophotometry at 418 nm. Linear calibration was obtained with 9.4–46.9 μg ml^{-1} with a detection limit of 4.7 ng ml^{-1}. Vitamin B_{12} and rifampicin when present together with PPA separated completely and could be determined simultaneously. PPA was determined in pharmaceutical preparations with a relative standard deviation of 0.6–1.6%. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Phenomenex C-18; Methanol-water; Rifampicin

1. Introduction

Phenylpropanolamine (PPA) is a sympathomimetic agent with vasoconstrictor and decongestant effects on inflamed mucous membranes. It is also reported as an appetite suppressant. A number of analytical methods have been reported for the determination of PPA mostly based on spectrophotometry [1–6], spectrofluorimetry [7], room temperature phosphoresence [8], fluoroimmunoassay [9], radioenzymic assay [10] Raman spectroscopy [11], thin layer [12], gas [13–17] and liquid chromatography [18–44]. For liquid chromatography a reversed phase, reversed phase ion pair or cation exchanger has been reported without derivatization or with pre- and post-column derivatization procedures. The detection is obtained by spectrophotometer or spectrofluorimeter. Precolumn derivatization procedures increase the sensitivity for the spectrophotometric detection of PPA. For precolumn derivatization 3,5-dinitrophenylisocyanate [24], 9-fluorenylmethylchloroformate [25], 4-fluoro-

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7-nitrobenzofurazan [32], *o*-phthalaldehyde [33], and phenylisothiocyanate [40] have been reported using spectrophotometric and spectrofluorometric detection. The detection limits have been reported at ng ml^{-1} levels. In an attempt to develop an analytical method for PPA using an economical derivatizing reagent, different reagents were examined, but 4-dimethylaminobenzaldehyde (DAB) is a common derivatizing reagent for compounds containing primary amino groups and generally a significant bathochromatic shift is observed in the derivatives to monitor in the visible region [45].

It eliminates the possible interference from a number of pharmaceutical products absorbing in the UV region. DAB was therefore examined for the HPLC determination of phenylpropanolamine (PPA) using spectrophotometric detection in the visible region Fig. 1.

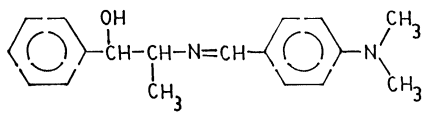

Fig. 1. Structural diagram of DAB derivative of PPA.

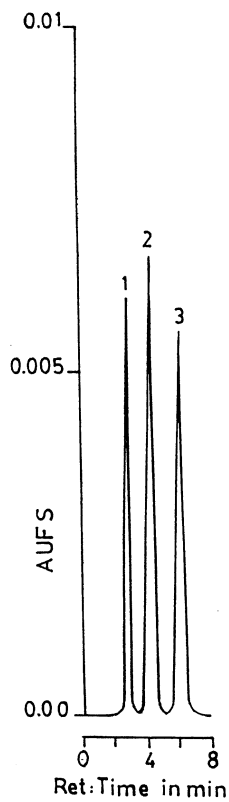

Fig. 2. HPLC separation of (1) vitamin B_{12}; (2) DAB-PPA; (3) rifampicin on phenomenex C-18 (150×4.6 mm i.d.) and eluted with dioxane/water/methanol (20:30:50, by vol.) with a flow rate of 1 ml min^{-1}. Spectrophotometric detection was at 418 nm.

2. Experimental

4-Dimethylaminobenzaldehyde (BDH) phenylpropanolamine hydrochloride (Sandoz Pak) Vitamin B_{12} (cyanocobalamin) (Fluka) rifampicin (Abbott, Pak, Karachi) methanol (E. Merck), dioxane (M&B), Chloroform (E. Merck), sodium hydroxide (Fluka) were used. Spectrophotometric studies were carried out using a Hitachi 220 spectrophotometer. HPLC studies were carried out on a Varian 9010 solvent delivery system, connected with a Varian 9050 Variable wavelength UV/Vis detector, Rheodyne 7161 injector and Varian 4400 integrator. A phenomenex 1B-SIL 5C18 (150×4.6 mm i.d.) (Torrance, CA, USA) column was used throughout the study.

2.1. Quantitative spectrophotometric procedure

An aqueous solution (1–5 ml) containing phenylpropanolamine hydrochloride (PPA.HCl) (112–450 μg) was transferred to a separating funnel and sodium hydroxide (0.5 ml, 0.2% w/v) was added. Chloroform (4 ml) was added and the contents were thoroughly mixed. The layers were allowed to separate and the organic layer was collected in a 25-ml volumetric flask. The extraction was repeated with chloroform (4 ml) and to the combined extracts was added 4-dimethylaminobenzaldehyde (DAB) (2 ml, 0.5% w/v in methanol) and acetic acid (0.5 ml). The contents were heated on a water bath at 80–90°C for 15 min and the volume was adjusted to the mark with methanol. The absorption spectrum was recorded in the visible region within 350–500 nm against a reagent blank. The aqueous layer re-

Table 1
Analysis of phenylpropanolamine (PPA) in pharmaceutical preparations by spectrophotometry

Sr no.	Name of preparation	Name of drug present	Amount reported (mg/tablet)	Amount found by spectrophometer mg/tablet (R.S.D.%)[a]	%RD[b]
1	Tavegyl-D	Clemastine (as hydrogen fumarate)	0.1	–	–
		Phenyl propanolamine hydrochloride	75	72.3 (0.78)	3.6
2	Sinutab	Paracetamol	325	309 (0.77)	4.9
		Phenyl propanolamine hydrochloride	25	24.0 (0.4)	0.4
		Phenyl toloxamine citrate	22	–	–
3	Panadol	Paracetamol	500	475 (0.65)	4

[a] R.S.D. = Relative Standard Deviation.
[b] RD = Relative Deviation.

Table 2
HPLC analysis of phenylpropanolamine (PPA) in pharmaceutical preparations

Sr no.	Name of preparation	Name of drug present	Amount reported (mg/tablet)	Amount found by HPLC mg/tablet (R.S.D.%)[a]	%RD[b]
1	Tavegyl-D	Clemastine (as hydrogen fumarate)	0.1	–	–
		Phenyl propanolamine hydrochloride	75	71.81 (0.6)	4.25
2	Sinutab	Paracetamol	325	–	–
		Phenyl propanolamine hydrochloride	25	23.84 (1.2)	4.64
		Phenyl toloxamine citrate	22	–	–

[a] R.S.D. = Relative Standard Deviation.
[b] RD = Relative Deviation.

maining in the separatory funnel was collected in a 25-ml volumetric flask and the volume was adjusted to the mark with water. The absorbance was measured at 291 nm against water for paracetamol determination.

2.2. HPLC determination

A solution (1–5 ml) containing PPA (37.5–187.5 μg) was added to sodium hydroxide (0.5 ml, 0.2% w/v in water) and chloroform (2 ml). The

contents were mixed well and the layers were allowed to separate. Exactly 1 ml of chloroform was taken from the organic layer and DAB (1 ml, 0.5% w/v in methanol) and acetic acid (0.1 ml) were added. The contents were heated on a water bath at 80–90°C for 10 min and the final volume was adjusted to 2 ml. The solution (10 μl) was injected on to a phenomenex C_{18} 5 μm (150 × 4.6 mm i.d.) column and eluted with dioxane/water/methanol (20:30:50, by vol.) with a flow rate of 1 ml min^{-1}. The spectrophotometric detection was at 418 nm.

2.3. Analysis of phenylpropanolamine hydrochloride in pharmaceutical preparations

Ten tablets each of Tavegyl-D (Sandoz (Pak) Ltd. Karachi) and Sinutab (Parke-Davis & Co. (Pak) Ltd. Karachi) were ground. Powder (0.051 g) from Travegyl-D and 0.0475 g from Sinutab tablets were separately dissolved in water by warming on a water bath at 70–80°C. The solutions were filtered and the final volume was adjusted to 50 ml with distilled water. For the spectrophotometric determination, a solution (2 ml) of Tavegyl-D or (4 ml) from the Sinutab tablets was transferred to a separatory funnel and the procedure was followed as in Section 2.1. The absorbance was measured at 397 nm. The amount of PPA in pharmaceutical preparations was evaluated from the calibration curve prepared with pure PPA. For the HPLC determination, a solution (0.5 ml) of Tavegyl-D or (1 ml) from Sinutab was taken and the solution was processed as in Section 2.2. The amount of PPA in the pharmaceutical preparations was evaluated from an external calibration curve prepared from standards of pure PPA.

3. Results and discussion

Phenylpropanolamine (PPA) absorbs only weakly in the UV region at 210 and 256 nm with a molar absorptivity of 3778 and 156 l mol^{-1} cm^{-1}. In order to increase the spectrophotometric sensitivity for the determination of PPA, a derivatization process with DAB was carried out. Initially derivatization in aqueous methanolic media was carried out with and without addition of hydrochloric acid. The derivatization was also carried out in the presence of sodium hydroxide. Each time quantitative derivatization was not observed, with a deviation from the linear relationship with change in the concentration of PPA. The amine PPA was extracted from alkaline media into chloroform and the derivatization was carried out in chloroform methanolic media, in the presence of acetic acid. Effective derivatization was indicated with an increase in absorbance with increase in the concentration of PPA. The maximum absorbance of PPA derivative was observed at 397 nm with molar absorptivity of 19 069 l mol^{-1} cm^{-1} as compared to DAB at 342 nm with molar absorptivity of 31 758 l mol^{-1} cm^{-1}. The change in absorbance with time was investigated. The absorbance of the solution after derivatization and at different time intervals was recorded. It was observed that any change in absorbance up to 24 h was not recorded.

Beer's law was obeyed for the concentration of 4.5–18.0 μg/ml of PPA using the derivatization procedure. The coefficient of correlation (r^2) for the calibration curve with five standards ($n = 5$) was observed to be 0.9996 with the regression equation $Y = 0.059X - 0.0043$. Possible additives, such as lactose, gum accacia, methylparabin, propyl parabin, sorbitol, propylene glycol did not affect the quantitative response of PPA when added at a concentration of 10 times that of PPA with a relative standard deviation within ± 2.9 ($n = 10$). The spectrophotometric method gave reasonable sensitivity, but an alternative HPLC method based on precolumn derivatization with DAB and elution from reversed phase column and detection by spectrophotometry in the visible region was examined. PPA derivative easily eluted from the phenomenex C-18 column (150 × 4.6 mm i.d.) when eluted with methanol–water. When the wavelength of the spectrophotometric detection was fixed at 397 nm, at the maximum absorbance of PPA derivative some response of derivatizing reagent DAB was observed due to incomplete separation of DAB from the PPA derivative. However, when the wavelength was shifted to 418 nm, the excess of the derivatizing

reagent DAB added during the derivatizing procedure did not show any response, with only a minor effect in decreasing the sensitivity of PPA derivative and the detection was selected.

In the pharmaceutical preparations clemestine as hydrogen fumerate and phenyltoloxamine citrate are commonly present in combination with PPA, but clemestine and phenyl toloxamine citrate absorb in the UV region and did not show any response at the selected wavelength (418 nm) for the detection of PPA derivative. Cyanocobalamin (vitamin B_{12}) and rifampicin used as anti pernicious anemia factor and as antituberculosis agents, respectively, are colored compounds and indicated some absorbance at the selected wavelength for the detection of PPA derivative. Therefore the compounds were examined for the HPLC separation from PPA derivative. Different binary and ternary solvent systems were examined, but an optimal separation was obtained with methanol/water/dioxane (50:30:20, by vol.) with a flow rate of 1 ml min^{-1}. Complete separation was obtained with a resolution factor (R_s) between two adjacent peaks >3 with elution of vitamin B_{12} first, followed by PPA derivative and rifampicin (Figs. 1 and 2). Using these conditions, the effect of concentration of PPA on the average peak height ($n=3$) was examined and the linear calibration curve was obtained with 9.4–46.9 μg/ml corresponding to 93.8–468.8 ng per injection (10 μl) with a coefficient of correlation (r^2) of 0.9998 and $Y=0.3005X-0.076$. Reproducibility of the response from the solution containing 18.4 μg/ml was checked and the average peak height or peak area ($n=5$) gave a R.S.D. within $\pm 2\%$.

The detection limits, measured as three times the background noise for PPA, vitamin B_{12} and rifampicin, were 11.7 ng ml^{-1}, 0.34 μg ml^{-1} and 16.6 μg ml^{-1} corresponding to 117 pg, 3.4 ng and 166 ng per injection (10 μl), respectively. The pharmaceutical preparation Tavegyl-D and Sinutab were analyzed for the contents of PPA using both spectrophotometric and HPLC methods. Ten tablets of each sample were taken and after grinding, mixing and sub-dividing, a small portion of the sample was taken and processed for analysis. The results (Tables 1 and 2) indicated an R.S.D. of 0.4–0.78 for spectrophotometric and 0.6–1.2% for HPLC methods.

4. Conclusion

An analytical method has been developed for the determination of phenylpropanolamine (PPA) by spectrophotometry and liquid chromatography coupled with spectrophotometric detection. 4-Dimethylaminobenzaldehyde was used as a derivatizing reagent. A quantitative response was obtained with a calibration range at μg/ml levels. It was possible to separate PPA from vitamin B_{12} and rifampicin by reversed phase HPLC.

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