Original Research Article

New Spectrophotometric Methods for the Determination of p-Aminosalicylic Acid in Tablets

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Abstract

Purpose: To develop a new spectrophotometric method with improved sensitivity and at higher wavelength for the determination of p-aminosalicylic acid in tablets.

Methods: Two simple and sensitive spectrophotometric methods (methods A and B) were developed using p-dimethylaminobenzaldehyde (DAB), and p-dimethylaminocinnamaldehyde (DAC) as derivatizing reagents for the determination of p-aminosalicylic acid (PAS) in tablets. The derivatization was carried out using 3M HCl-KCl buffer for DAB and 5M HCl-KCl buffer solutions.

Result: The new derivatives of PAS absorbed maximally with bathochromic shift to 460 and 555 nm in the linear concentration range of 0.4 – 2.0 µg/mL with molar absorptivities of 2.4×10^4 and 3.8×10^4 L/mole/cm, respectively, compared to pure PAS which absorbed at λ_{max} of 264 nm with molar absorptivity 7.65×10^3 L/mole/cm in the linear concentration range of 2 - 10 µg/mL. The developed methods were successfully applied to assay PAS in tablets with % recovery of 97.6 ± 1.71 and 98.4 ± 1.45 for methods A and B, respectively.

Conclusion: Both PAS derivatives absorb in the visible spectral region. The presence of excipients in pharmaceutical preparations did not interfere in the determination of PAS as PAS-DAB and PAS-DAC derivatives. Both methods can be applied to determine PAS from bulk and various pharmaceutical dosage forms.

Keywords: p-Aminosalicylic acid, p-dimethylaminobenzaldehyde, p-dimethylaminocinnamaldehyde, Spectrophotometry

INTRODUCTION

ρ-aminosalicylic acid (PAS) or 4-amino-2-hydroxybenzoic acid is an anti-tubercular drug and is also used in the treatment of ulcerative colitis [1]. The review of literature reported only a few spectrophotometric [2,3], fluorometric [4], flow injection analysis [5], thin layer chromatography (TLC) [6], high performance liquid chromatography (HPLC) [7,8], nuclear magnetic resonance (NMR) [9], titrimetric [10], polarographic [11], micro-dialysis [12], radioimmune assay [13], capillary electrophoretic

[14] and ion exchange chromatographic [15] techniques for the quantitative estimation of PAS in bulk, pharmaceutical formulations and in biological samples. In view of the above fact, some simple analytical methods are in need for its quantitative estimation.

Lianidou *et al* [4] used benzoyl-L-tyrosyl-PABA/PAS test. PAS was measured as a ternary complex with terbium and EDTA. A fluorescent compound was obtained with absorption at 324 nm. The apparent molar absorptivity of complex has not been mentioned and Beer's law was

obeyed over the range 1 - 6 µg/mL. Blake *et al* [15] determined PAS concentration 5 - 18 mg/mL using ion exchange chromatography and Vetuschi *et al* [2] determined PAS 10 - 40 µg/mL. The present study has been carried out to develop new methods for the determination of PAS by reacting with p-dimethylaminobenzaldehyde (DAB), and p-dimethylaminocinnamaldehyde (DAC) reagents with improved molar absorptivities of 2.4×10^4 and 3.8×10^4 L/mole/cm than above reported methods. Further in this study the new derivatives of PAS absorb maximally with bathochromic shift to 460 and 555 nm with concentration range 0.4 – 2.0 µg/mL as compared to already reported methods.

EXPERIMENTAL

Instrumentation

Double beam spectrophotometer (UV/Visible spectrometer (Lambda 25, Perkin-Elmer, USA) with dual silica 1 cm cuvettes was used for absorbance measurements. The spectrophotometer was controlled by A computer with UVWinLab 25 software. The pH of various buffers was monitored using a pH meter (Schot Instrument Lab 850, Germany).

Materials and reagents

All the chemicals and reagents used were of analytical or pharmaceutical grades. The double distilled water used throughout the study was obtained from distillation plant all made of glass. Pure ρ-aminosalicylic acid (PAS), ρ-dimethylaminocinnamaldehyde (DAC) and sodium acetate were obtained from Fluka (Switzerland). ρ-dimethylaminobenzaldehyde (DAB), Acetic acid and ethanol were purchased from E. Merck, (Germany). Buffer solutions between pH 0.5-10 at unit internal were prepared from the solution of (1-6 M) hydrochloric acid-potassium chloride (pH 1-2), acetic acid-sodium acetate (pH 3-7), sodium bicarbonate-sodium carbonate (pH 8-9) and ammonium chloride and ammonia solution (pH 10-11).

Method A

1. DAB (2 %) was prepared by dissolving 2 g of DAB in 100 mL of ethanol.
2. pH 0.5 (3 M HCl- KCl).

Method B

1. DAC (2 %) was prepared by dissolving 2 g of DAC in 100 mL of ethanol.

2. pH 0.5 (5 M HCl-KCl).

Preparation of standard and sample solution

Accurately weighed 0.01 g of PAS was dissolved in 100 mL of water. The final concentration was brought to 5 µg/mL of PAS for both methods A and B.

Assay procedures

Method A

Aliquots of standard drug solution ranging from 0.4 - 2 mL PAS solution (5 µg/mL) were taken separately into 5 mL volumetric flasks and then ethanolic solution of DAB (2 %) was added followed by 0.5 mL buffer of pH 0.5 (3 M HCl-KCl). The solutions were finally made up to the mark with distilled water and kept at room temperature (25 °C) for 5 min. The absorbance of the solution was measured at λ_{max} 460 nm against the corresponding reagent blank. The amount of PAS was computed from the linear regression equation.

Method B

Aliquots of standard drug solution ranging from 0.4 - 2 mL of PAS (5 µg/mL) were transferred to a series of 5 mL calibrated volumetric flasks. To each volumetric flask, 1 mL of DAC (2 % ethanolic) and 0.5 mL of buffer pH 0.5 (5 M HCl-KCl) were added and then kept aside for 30 min at room temperature (25 °C). The solutions were then finally made up to the mark with ethanol and the absorbance of the solution was measured at λ_{max} 555 nm against the corresponding reagent blank. The amount of PAS was computed from the Beer's Lambert's plot.

Assay PAS in tablets

Ten tablets of two products from different pharmaceutical companies were collected for the analysis of PAS. An accurately weighed amount of tablets equivalent to 0.01 g of PAS for each preparation namely PASK (Akrikhin Pharmaceuticals – Moscow, Russia) and PAS (Star Laboratories (PVT). LTD., Lahore, Pakistan), was dissolved in the 100 mL volumetric flask containing sufficient volume of distilled water by shaking well and then filtered through Whatman No. 1 filter paper before adjusting to the final volume (100 mL) with water. This solution was considered as the stock solution. Convenient aliquots of stock solution were taken for the determination of PAS and the procedures were repeated as described for methods A and B.

The effect of adding 0.5-2.0 mL of DAB and DAC (2 % ethanolic) reagent solutions with an intervals of 0.5 mL were examined on absorbance of 0.8 µg/mL PAS (5 µg/mL). The absorbance was measured at λ_{max} 460 and 555 nm, respectively. Same absorbance was observed with addition of 1.5 mL and above for DAB and 1 mL and above for DAC.

The effect of time on the formation of derivatives in terms of absorbance of 1.2 µg/mL PAS solution in the presence of 2 % ethanolic DAB and DAC solutions was examined at 460 and 555 nm from 0-30 min with an interval of 5 min at 60 - 90 °C in a water bath. The absorbance of both derivatization procedures was also measured at room temperature (25 °C) from 0 - 30 min at an interval of 5 min.

Statistical analysis

Paired t-test was applied to compare the mean values of analysis of pharmaceutical preparation with labeled values at 95 % confidence level. Student t-test was applied to calculate confidence interval at 95 % for the analyzed drug. Statistical calculations were carried out using SPSS 16.0 software package.

RESULTS

ρ-Aminosalicylic acid (PAS) or 4-amino-2-hydroxybenzoic acid reacts with DAB and DAC to form an azomethine derivatives 4-[(3-Dimethylamino-benzylidene)-amino]-2-hydroxy-benzoic acid (PAS-DAB) and 4-[3-(4-Dimethylamino-phenyl)-allylideneamino]-2-hydroxy-benzoic acid (PAS-DAC), respectively (Figure 1). The molar absorptivity of pure PAS was calculated as 7.65×10^3 L/mole/cm at λ_{max} 264 nm and obeyed the Beer's law within 2 - 10 µg/mL. The method A is based on the reaction of PAS with ρ-dimethylaminobenzaldehyde (DAB). The molar absorptivity of resulting colored chromogen was calculated as 2.4×10^4 L/mole/cm at λ_{max} 460 nm. Method B is based on the reaction of PAS with ρ-dimethylaminocinnamaldehyde (DAC) to form a colored solution with molar absorptivity of 3.8×10^4 L/mole/cm at λ_{max} 555 nm. Beer's law was obeyed in the concentration range of 0.4 - 2.0 µg/mL for both methods.

The maximum absorbance of PAS-DAB and PAS-DAC derivatives were observed at 0.5 mL buffer pH 0.5 of 3 M HCl-KCl for DAB and 5 M HCl-KCl for DAC, respectively (Figure 4).
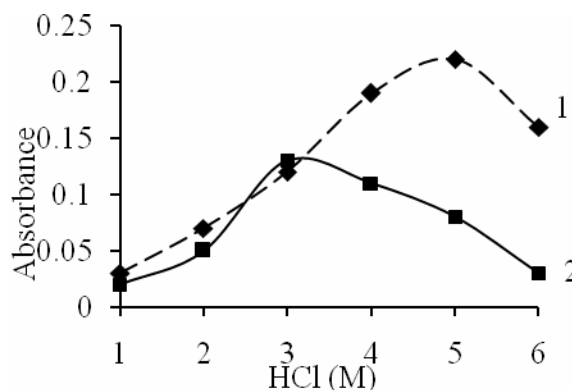
1. PAS-DAB, 2. PAS-DAC

Figure 4: Optimization of pH

The effect of possible presence of different pharmaceutical additives such as sodium chloride, mannitol, sorbitol, lactose, sucrose, glucose, galactose and fructose was investigated at 10 times the concentration of PAS and it was observed that none of these substances interfered when the absorbance was changed within ± 4 %.

The absorbances of the solutions were measured at different time interval after formation of derivatives. There was no significant change in absorbance was observed for both PAS-DAB and PAS-DAC derivatives with relative error within ± 4 % and the solutions were stable for more than 24 h at 25 °C.

Maximum absorbance was achieved at 264 nm for pure PAS and at 460 nm and 555 nm for PAS-DAB and PAS-DAC derivatives, respectively, against reagent blank. These wavelengths were selected as optimum for both derivatives (Figure 2).

Therefore, the addition of 1.5 mL of DAB and 1 mL of DAC solutions was selected (Figure 3).

The order of adding reagents during derivatization process played an important role in the accuracy of results and enhancement of absorbance. It was observed that the addition of 0.5 mL buffer (pH 0.5 of 3 M HCl-KCl for DAB and 5 M HCl-KCl for DAC to PAS solution (0.8 mL) followed by the DAB and DAC reagent (ethanolic, 2 %) resulted in a decrease in absorbance value. Taking the reagent first and then adding the buffer, followed by PAS solution also decreased the absorbance value. Maximum absorbance value was observed when optimum
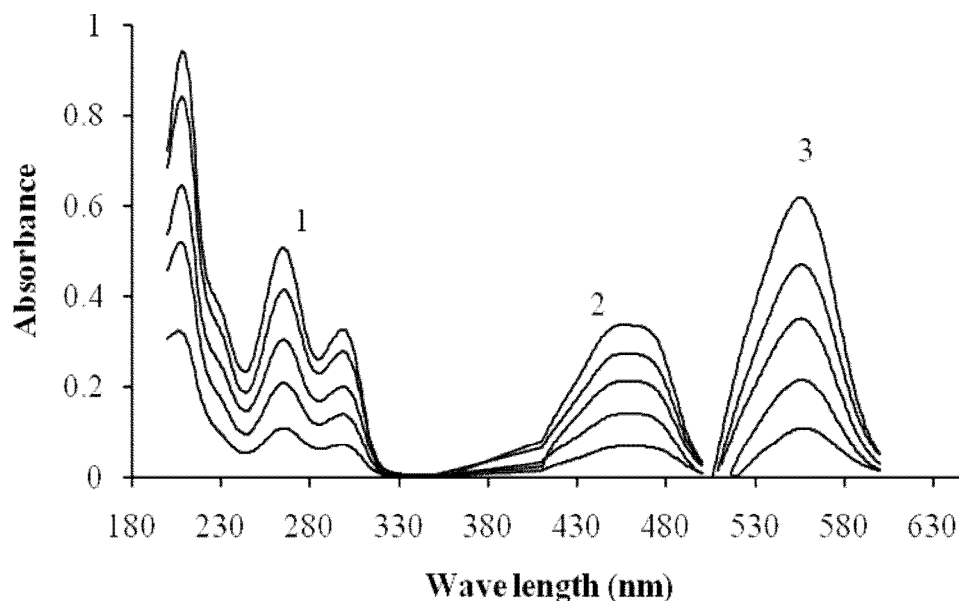
Figure 2: Optimization of analytical wavelength. ***Note:*** 1 = pure PAS (264 nm); 2 = PAS-DAB (460 nm); 3 = PAS-DAC (555 nm)
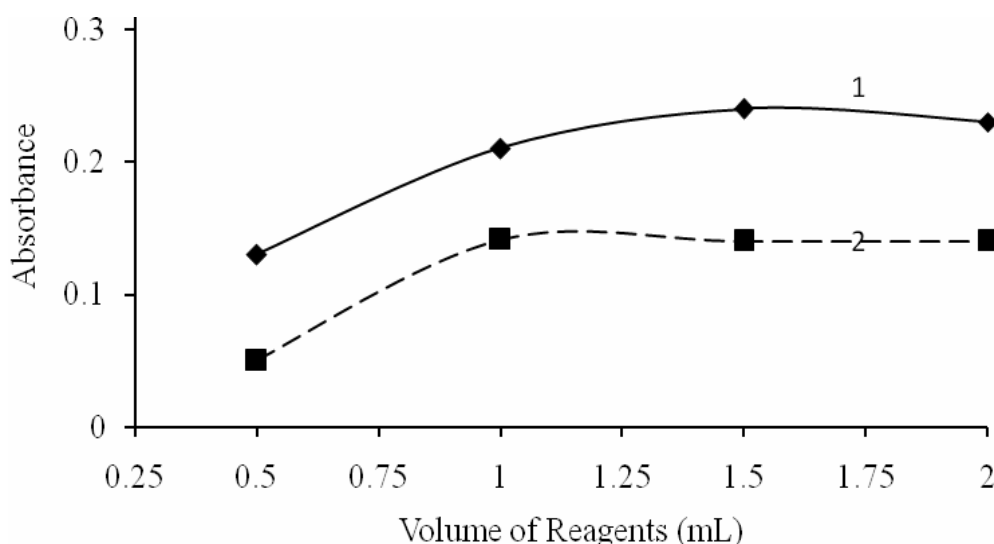

Figure 3: Optimization of volume of reagents (1 = DAB, 2 = DAC)

volumes of DAB and DAC were added to the aliquots of PAS, followed by 0.5 mL buffer (pH 0.5 of 3 M HCl-KCl for DAB and 5 M HCl-KCl for DAC. The contents were then kept aside at room temperature (25 °C) for 0 and 30 min and the volumes adjusted to 5 mL with water and ethanol.

Calibration plots

The effect of variation in the concentration of PAS on its absorbance was studied. The linear calibration curves were obtained which obeyed the Beer's law within the concentration range 0.4

- 2.0 µg/mL of PAS with coefficient of determination (R^2) of 0.9997 for both methods A and B. Sandell's sensitivity (A = 0.004) was observed at 0.03 and 0.015 µg/mL for PAS-DAB and PAS-DAC derivatives, respectively. The validity of the calibration curves was obtained by the analysis of test solutions of PAS (n = 5) and % relative error was ± 0.97. Recovery, calculated by standard addition technique, was within the range 98 – 100 % with RSD of 1.7 and 1.4 % for methods A and B. The tablets containing PAS available in the local market were analyzed to determine the amount of PAS quantitatively. The mean values (g) and range of % error (95 %

Table 1: Optical characteristics

Parameter	Method	
	a*	**b***
Beer's law limits (µg/mL)	0.4-2.0	0.4-2.0
λ_{max} (nm).	460	555
Molar absorptivity (L/mol/cm)	2.4×10^4	3.8×10^4
Sandell's sensitivity (µg/mL at 0.004 absorbance unit)	0.03	0.015
Regression equation (y)[a]		
Intercept (a)	0	0
Slope (b)	0.1644	0.2877
Coefficient of determination	0.9997	0.9997
Relative standard deviation (± %) 1g	0.03	0.023
Relative standard deviation (± %) 0.5g	0.69	0.11
Relative deviation (± %) 1g	2	1
Relative deviation (± %) 0.5g	1	0.1
Mean value, 1 g/tablet ± range of error % at 95 % confidence limit	0.98 ± 0.94	0.99 ± 0.46
Mean value, 0.5 g/tablet ± range of error % at 95 % confidence limit	0.49 ± 0.97	0.499 ± 0.58

a* = PAS-DAB, b* = PAS-DAC

Table 2: Intra- and inter-day accuracy and precision of PAS-DAB and PAS-DAC

Day	Absorbance (±%RSD)		% Relative error	
	a*	**b***	**a***	**b***
Inter-day	0.13(0.2)	0.22(1.0)	-1.2	0.5
Intra-day	0.14(2.8)	0.24 (0.3)	2	0.8

RSD = relative standard deviation; a* = PAS-DAB; b* = PAS-DAC

confidence limit) of PAS tablets was 0.49 ± 0.97 and 0.99 ± 0.46, respectively (Table 1) and no significant difference in the absorbance of PAS derivatives was found at 95 % confidence limit, compared with labeled value.

Inter-day reproducibility/repeatability

For the determination of intraday and inter-day reproducibility of the methods, the aqueous standard solution (0.8 mL) of 5 µg/mL PAS solution was taken in three different calibrated volumetric flasks (5 mL) and the procedures were followed as described in assay procedures. The absorbances were measured against reagent blank at 460 and 555 nm. The above procedures were repeated for three days (n=3). The mean absorbance of intraday and interday reproducibilities for PAS-DAB was 0.14 and 0.13, respectively, whereas for PAS-DAC, the values were 0.24 and 0.22, respectively (Table 2).

DISCUSSION

Different parameters including effects of pH, amount of reagents added, heating time, temperature and solvents on the formation of PAS-DAB and PAS-DAC derivatives were thoroughly studied.

In each spectrophotometric quantization, the λ_{max} has a profound position, so it is important that pure analyte (PAS) and derivatizing reagents (DAB and DAC) should not absorb light near the region where the analyte derivatives absorb. This may cause error in the absorption of the drug because the derivatizing reagents are added in excess to complete the reactions quantitatively. To get rid of this problem, the wavelength must be selected where the derivatizing reagent show least absorbance and the analyte derivative indicates optimum absorbance value.

The maximum absorbance of colored chromogens was observed after keeping PAS derivatives at room temperature (25 °C) for 0 - 30 min. Heating caused the absorbance of relevant derivatives to decrease. Therefore, heating was avoided for both PAS derivatives.

Water and ethanol were found to be the best among the solvents studied because these produced maximum absorbance and stability of PAS-DAB and PAS-DAC derivatives.
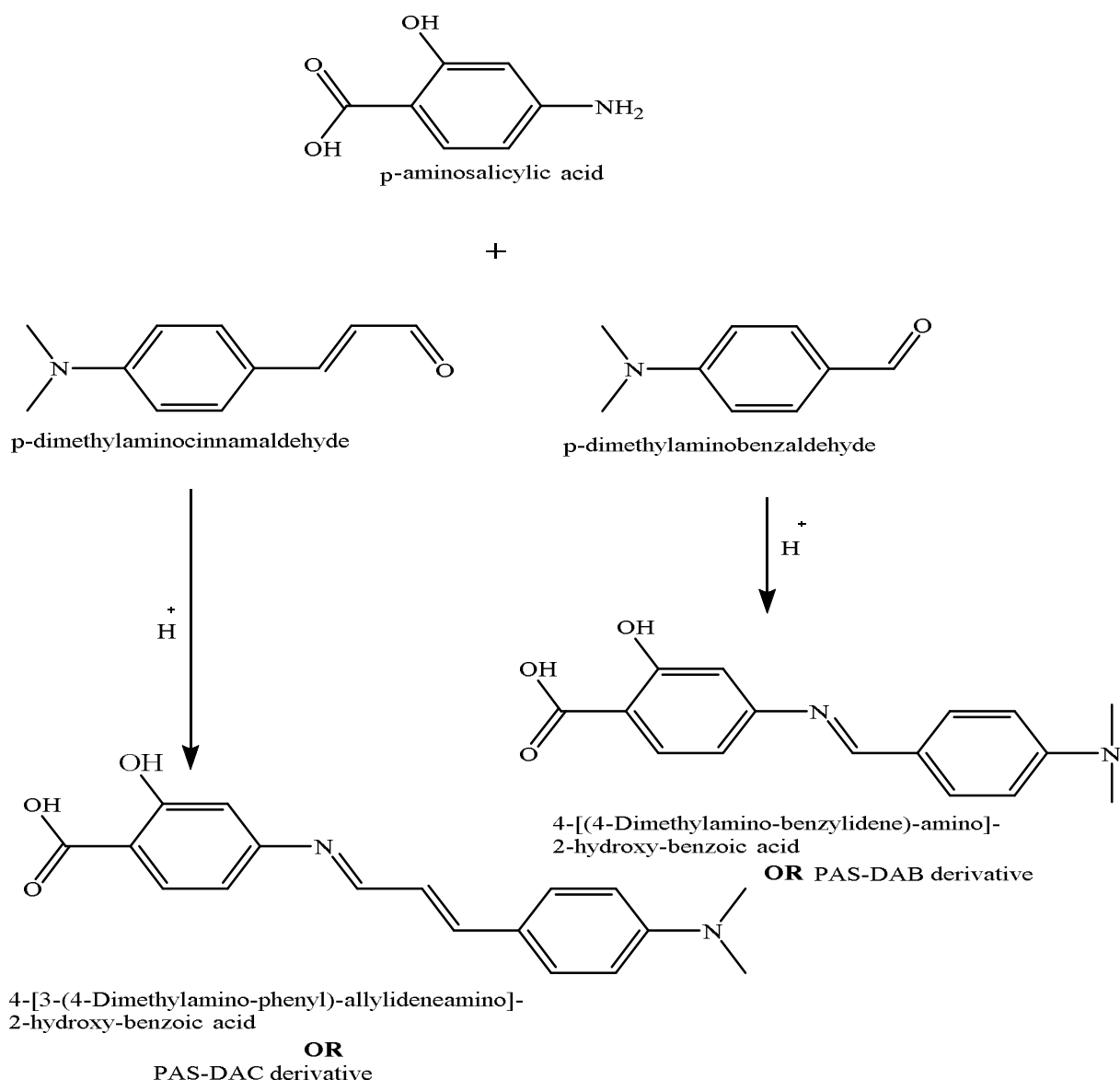
Figure 1: Formation of PAS-DAB and PAS-DAC derivatives using DAB and DAC as derivatizing reagents

CONCLUSION

The methods are free from the interferences of associated materials which may absorb in the UV region. The developed methods are simpler, and more sensitive and cost-effective than a previously reported spectrofluorometric method [4]. The methods are suitable for the determination of PAS in tablets.

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