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Determination of *p*-aminosalicylic acid and *m*-aminophenol by derivative UV-spectrophotometry

C. VETUSCHI,*‡ G. RAGNO* and P. MAZZEO†

* *Dipartimento Farmaco-Chimico dell'Università, Via Amendola 173, 70126 Bari, Italy*
† *Dipartimento di Chimica, Ingegneria Chimica e Materiali dell'Università, Via Assergi 4, 67100 L'Aquila, Italy*

Abstract: A direct method for the simultaneous determination of *p*-aminosalicylic acid (PAS) and its major decomposition product, *m*-aminophenol (MAP), is described. The analysis is based on the use of derivative UV-spectrophotometry and is a rapid procedure which gives accurate and precise results. A simple purity test which utilizes the third derivative spectrum is also reported and compared with the USP XX spectrophotometric method for the estimation of MAP in PAS formulations.

Keywords: *Derivative UV-spectrophotometry;* p-*aminosalicylic acid impurities;* m-*aminophenol; higher-order derivatives.*

Introduction

p-Aminosalicylic acid (PAS) is still frequently utilized as a therapeutic agent against tuberculosis, particularly as the sodium or calcium salt. The major decomposition product, *m*-aminophenol (MAP), formed by decarboxylation, is a toxic compound. Therefore the determination of MAP in PAS pharmaceutical formulations is clearly of importance for drug quality control.

The colorimetric limit test reported by the Italian [1], British [2] and European Pharmacopoeias [3] for the estimation of MAP in solid formulations containing the sodium or calcium salt of PAS is based on the oxidative coupling with 4-amino-*N*,*N*-diethylaniline. This reaction, initially described by Hrdý and Petříková [4], has recently been reinvestigated by Mazzeo-Farina and co-workers [5]. In the United States Pharmacopeia the MAP determination involves the spectrophotometric measurement of the product after reaction with sodium nitrite [6].

Previously, Shih [7] proposed a method for the colorimetric determination of MAP in PAS using *p*-nitrosobenzoic acid and Moussa [8] described a method for the selective spectrophotometric determination of PAS in the presence of MAP, without analysing for this impurity. Some HPLC procedures have also been reported for the determination of PAS and MAP [9], as impurities in formulations containing isoniazid, and for the estimation of MAP in PAS [10, 11].

‡To whom correspondence should be addressed.

In this paper a very simple, rapid accurate and precise method is reported for the direct simultaneous determination of PAS and MAP in bulk material and in pharmaceutical formulations containing PAS or its salts by derivative UV-spectrophotometry, which has proved to be of great value in eliminating the interference from impurities and coformulated drugs [12].

Experimental

Reagents and chemicals

Ethanol was spectroscopic reagent grade. *m*-Aminophenol and hydrochloric acid were analytical reagent grade. *p*-Aminosalicylic acid was purified by several recrystallizations of the commercial material from ethanol without heating or exposure to light.

Apparatus and conditions

The spectra were obtained with a Perkin–Elmer Model 320 ultraviolet-visible spectrophotometer. Data elaboration was by a Perkin–Elmer Data Station, Model 3500. Zero order spectra: scan speed 60 nm min^{-1}; spectral slit width 1 nm. Derivative conditions: scan speed 60 nm min^{-1}; spectral slit width 1 nm; $\Delta\lambda$ (third derivative bandwidth) 6 nm; $\Delta\lambda$ (fourth derivative bandwidth) 8 nm.

Standard solutions

Standard solutions were prepared in a mixture of absolute ethyl alcohol–HCl 10 M (90:10, v/v) with PAS concentrations in the range 10–40 $\mu g\ ml^{-1}$ and with MAP concentrations varying between 0.04 and 10 $\mu g\ ml^{-1}$. The PAS:MAP concentration ratio in the standard solutions is in the range $1–10^3$.

These solutions were utilized to establish the validity of the analytical methods developed for the determination of both substances.

Results

Effect of pH on the PAS and MAP ultraviolet spectra

Figure 1 shows a schematic form of the absorbance and wavelength values of the absorption maxima of aqueous alcoholic solutions of PAS and MAP at various pH values.

All the spectrophotometric determinations were carried out in the mixed solvent ethyl alcohol–water (90:10, v/v) adjusted to pH 0 with 10 M HCl. The stability of PAS solutions under these conditions, verified by the analytical procedure subsequently described, was found to be very high, since appreciable amounts of MAP were not formed in the time interval necessary for the determination.

PAS and MAP analysis

Figure 2 shows the zero-order ultraviolet spectra of PAS, MAP and its binary mixture; Figs 3 and 4 show the corresponding third- and fourth-derivative spectra. The convention proposed by Fasanmade and Fell [13] is used to represent the derivative amplitudes (usually measured in arbitrary units). Thus the leading superscript represents the derivative order, and the subscripts represent the wavelength of the positive and negative peaks measured, respectively.

The PAS determination in the presence of MAP in the concentration ranges examined

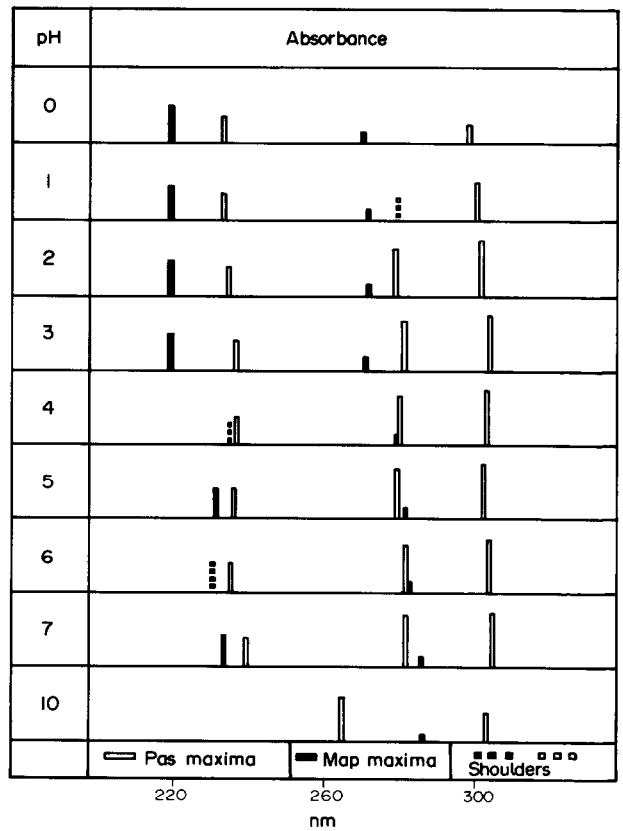

Figure 1
Scheme of absorbance and wavelength values of maxima of solutions of PAS (20 μg ml^{-1}) and MAP (20 μg ml^{-1}) in ethyl alcohol–water (90:10, v/v) at various pH values.

can be performed by measuring the absorption maximum at 300 nm in the zero-order spectrum, from the peak–trough amplitude between 306 and 314 nm ($^3D_{314,306}$) (h_3) in the third-derivative spectrum; it can also be based on the peak–trough amplitude between 310 and 318 nm ($^4D_{310,318}$) (h_4) in the fourth-derivative spectrum.

The MAP determination can be carried out by utilizing in the third-derivative spectrum the peak–trough amplitude between 226 and 234 nm ($^3D_{226,234}$) (h_3'), to which both substances contribute; this can then be corrected by a simple mathematical correlation for the contribution deriving from PAS (determined as previously described).

At the same time, the h_3'/h_3 ratio ($^3D_{226,234}/^3D_{314,306}$) can be used to determine the MAP/PAS concentration ratio in the sample and consequently its degree of purity.

Details of each of these methods are given below.

PAS determination. (a) The PAS determination is performed by measuring the absorbance at 300 nm in the zero-order spectrum and using the following linear equation obtained through regression analysis of data for the standard solutions previously reported:

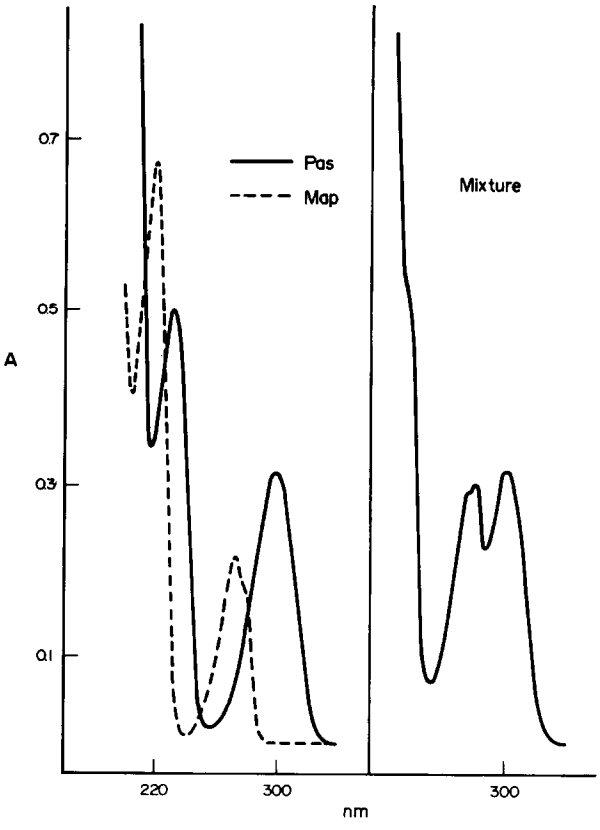

Figure 2
Zero-order ultraviolet spectra of PAS (10 μg ml^{-1}), MAP (10 μg ml^{-1}) and of a mixture of PAS (10 μg ml^{-1}) and MAP (10 μg ml^{-1}) in ethyl alcohol–water (90:10, v/v) at pH 0.

$$y = 0.030555x + 0.0031437 \text{ (correlation coefficient 0.99999)}$$

where y = absorbance and x = PAS concentration (μg ml^{-1}).

(b) From the amplitude of $^3D_{314,306}$ (h_3) in the third-derivative spectrum it is possible to obtain the PAS concentration by using the following equation, obtained through regression analysis of data for the standard solutions previously reported:

$$h_3 = 1.4482x + 0.12640 \text{ (correlation coefficient 0.99955)}$$

where $h_3 = {}^3D_{314,306}$ amplitude (measured in mm) on the scale ±1 absorbance units (AU), and x = PAS concentration (μg ml^{-1}).

(c) From the amplitude of $^4D_{310,318}$ (h_4) in the fourth-derivative spectrum it is possible to obtain the PAS concentration by utilizing the following equation, obtained through regression analysis of data for the standard solutions previously reported:

$$h_4 = 6.0936x + 0.97970 \text{ (correlation coefficient 0.99998)}$$

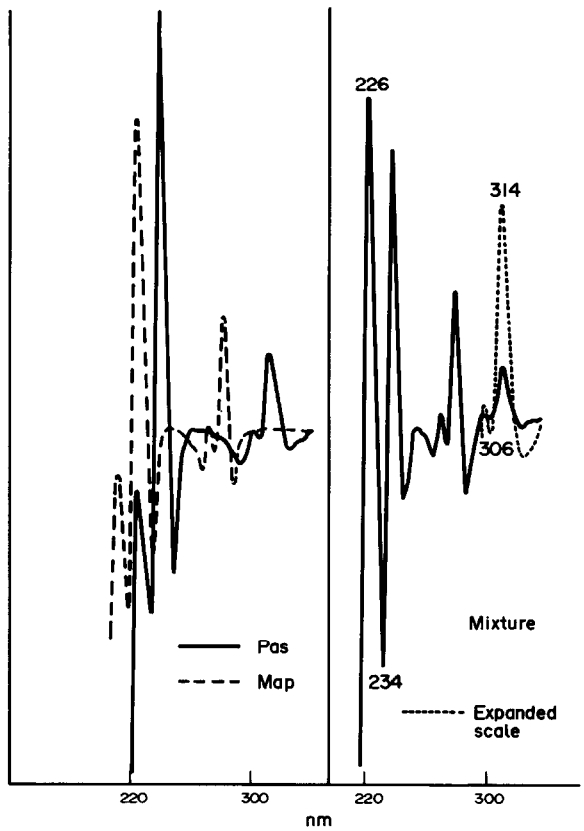

Figure 3
Third-derivative ultraviolet spectra of PAS (20 μg ml^{-1}), MAP (20 μg ml^{-1}) and of a mixture of PAS (10 μg ml^{-1}) and MAP (10 μg ml^{-1}) in ethyl alcohol–water (90:10, v/v) at pH 0.

where $h_4 = {}^4D_{310,318}$ amplitude (measured in mm) on the scale ±1 AU, and x = PAS concentration (μg ml^{-1}).

MAP determination. The total amplitude of $^3D_{226,234}$ (h'_3) in the third-derivative spectrum of various mixtures was measured. The contribution due to MAP only (h^*_3) was calculated, by subtracting from h'_3 the value (h'_{3PAS}) determined for PAS alone. The term h'_{3PAS} was derived using the following equation, obtained through regression analysis of data for standard solutions of PAS (5–45 μg ml^{-1}) under identical conditions:

$$h'_{3PAS} = 1.6465x + 0.36161 \text{ (correlation coefficient 0.99977)}$$

where h'_{3PAS} = graphical amplitude between points at 226 and 234 nm in the third-derivative spectrum produced by PAS alone (expressed in mm) on the scale ±1 AU, and x = PAS concentration (μg ml^{-1}), previously determined from the third-derivative spectrum.

The MAP concentration h^*_3 can then be obtained utilizing the following equation, obtained through regression analysis of data for the standard solutions previously reported:

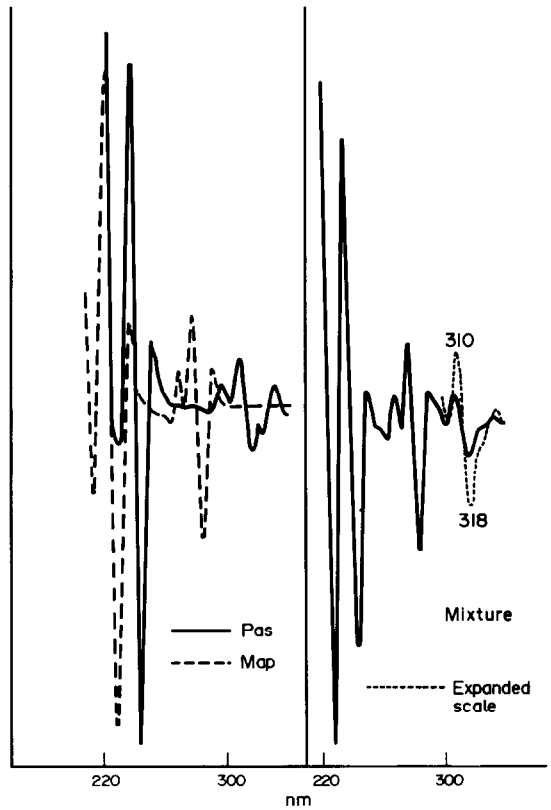

Figure 4
Fourth-derivative ultraviolet spectra of PAS (20 μg ml^{-1}), MAP (20 μg ml^{-1}) and of a mixture of PAS (10 μg ml^{-1}) and MAP (10 μg ml^{-1}) in ethyl alcohol–water (90:10, v/v) at pH 0.

$$h_3^* = h_3' - h_{3PAS}' = 13.0901y + 0.52277 \text{ (correlation coefficient 0.99997)}$$

where h_3' = $^3D_{226,234}$ amplitude (measured in mm) on the scale ±1 AU, h_3^* = net amplitude produced by MAP only, (expressed in mm) on the scale ±1 AU, and y = MAP concentration (μg ml^{-1}).

From the equations reported it is possible to derive the following expression, which gives the MAP concentration (y) as a function of the measured amplitudes h_3 ($^3D_{314,306}$) for PAS and h_3' ($^3D_{226,234}$) for MAP:

$$y = 0.07639h_3' - 0.08685h_3 - 0.05658.$$

MAP/PAS ratio determination. From the h_3'/h_3 ratio in the third-derivative spectrum it is possible to derive the MAP/PAS concentration ratio in the sample by using the following equation, obtained through regression analysis of data for the standard solutions previously reported:

$$h_3'/h_3 = 8.6320x + 1.15694 \text{ (correlation coefficient 1.00000)}$$

$$\text{where } x = \frac{\text{MAP concentration } (\mu g\ ml^{-1})}{\text{PAS concentration } (\mu g\ ml^{-1})}.$$

The MAP concentration can thus be obtained using the value of PAS concentration determined as previously described.

Sample analysis

The powdered pharmaceutical formulation or bulk material was extracted with a mixture of absolute ethyl alcohol–HCl 10 M (90:10, v/v) in such a way as to obtain a solution with a PAS concentration in the range 10–40 $\mu g\ ml^{-1}$. After filtration, the solution was analysed by the methods described.

Table 1 shows the results for the PAS and MAP determination in commercial PAS and in aminosalicylate sodium tablets in comparison with those obtained by the official USP XX (1980) procedure for MAP.

Discussion

The change in absorbance with pH for PAS and MAP, as shown in Fig. 1, allows the optimum pH for their simultaneous determination to be chosen. As found by Moussa [8], PAS absorbance decreases with fall in pH, but the λ_{max} values do not undergo significant change. For MAP, on the other hand, the absorbance rises with decreasing pH and the λ_{max} values shift appreciably towards shorter wavelength values. Thus it is evident that a very acidic medium represents the optimum condition for the determination of MAP in PAS dosage forms. At higher pH values, it is not possible to determine both substances simultaneously and only the PAS estimation, previously described by Moussa [8], can be carried out.

By using the proposed methods for the determination of PAS and MAP described, satisfactory results have been obtained, characterized by a high degree of accuracy (errors between 0 and 2.5%) and precision (relative standard deviation between ±0.2 and ±1.0% for a minimum number of five determinations).

Among the procedures proposed for the estimation of PAS, the use of the third-derivative spectrum appears to be preferable, even if characterized by a slightly lower accuracy than the methods based on zero-order and fourth-derivative spectra. Only this method allows the simultaneous determination of PAS and MAP. Similar conclusions were also found by other workers [13], who found that the third-derivative spectrum gave excellent discrimination against the interfering background, whereas the fourth-derivative spectrum was too noisy.

Of particular significance among the methods described is the use of the h_3'/h_3 ratio as a criterion of purity for PAS formulations. Unlike the official USP XX method which involves a laborious and time-consuming procedure, the use of the third-derivative spectrum is rapid and simple. The h_3'/h_3 ratio is directly proportional to the amount of MAP present in the PAS sample, with a value of 1.18 corresponding to 0.25% MAP, the level usually accepted as the PAS purity limit.

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Table 1
Results* obtained for the determination of PAS and MAP in raw material of different provenance and tablets, compared with the official USP XX procedure for MAP

Sample	Amount taken (mg)	PAS found (zero-order method) (mg)	PAS found (third-derivative method) (mg)	PAS found (fourth-derivative method) (mg)	MAP found (third-derivative method) (mg)	h_3'/h_3	MAP found (USP XX) (mg)
Commerical PAS no. 1	20.00	19.12	19.21	19.18	1.23	1.69	1.19
Commercial PAS no. 2	20.01	19.45	19.32	19.40	0.80	1.49	0.98
Commercial PAS no. 3	20.00	19.97	20.11	19.92	0.11	1.20	0.13
Tablets	20.06	19.46	19.58	19.50	0.13	1.21	0.11

* Expressed as the mean of 5 determinations for each sample.

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