

# Determination of *m*-Aminophenol (MAP) in *p*-Aminosalicylic Acid (PAS) and Sodium *p*-Aminosalicylate (PASNa)

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The MAP test in the U.S.P. XVI monographs for PAS and PASNa has been re-evaluated. Experimental data based upon paper chromatography showed the test to be sound but in need of minor changes to correct sources of error. As a result of this study, recommendations are made to change the correction factor, to determine maximum absorbance through a range of 420 to 435  $m\mu$ , and to change the time allowed for development of color to 3 hours.

THE MAP TEST in the U.S.P. XVI monographs for PAS and PASNa (1) is based upon a color development shown by Pesez (2) to be the result of a series of diazotization coupling reactions. Seaman, *et al.* (3), refined the method which Pesez had published. The color reactions are comprehensively reviewed by Feiser and Feiser (4). The U.S.P. XVI test for MAP is basically a sound analytical procedure; however, experience has indicated that certain discrepancies exist in the test. It has been recognized for some time that absorbance values in the MAP test often were less than 0.372 (the quantity alleged to represent color formed by PAS itself in the test), resulting in apparent minus percentage values for MAP content. Furthermore, the absorbance values decrease during the first 3 hours after color development possibly as the result of a decrease in background color. In an effort to understand the apparent variables in this test for MAP better, an investigation was undertaken to study the various conditions involved in the U.S.P. XVI test for MAP.

In order to establish the absolute MAP content of some production samples of PAS and PASNa, a paper chromatographic procedure was developed in our laboratory. Briefly, the procedure consisted of chromatographic separation of the MAP from the PAS and PASNa using a benzene and methanol mobile solvent and the quantitative estimation of MAP by a spectrophotometric ultraviolet scanning technique.

## EXPERIMENTAL

**Proposed Modified Colorimetric Method for MAP in PAS and PASNa.**—The proposed revisions of the U.S.P. XVI test for MAP, as described

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under *Procedures*, were performed on samples of PAS and PASNa. The basis for the revisions proposed are discussed under *Conditions Affecting the Colorimetric Determination of MAP*.

**Reference PASNa.**—The reference PASNa used in this investigation was a sample that was shown to contain less than 0.01% of MAP, as determined by the paper chromatographic method. When tested by the proposed revised colorimetric method described under *Procedures*, the reference PASNa showed an absorbance of 0.320. Repeating this test on the reference PASNa through a period of 20 different days, a range of absorbance values of 0.319 to 0.322 was obtained. In view of this, it was concluded that this sample represented reference quality PASNa in conjunction with this study.

A specially prepared sample of PASNa received from Dr. L. H. Welsh,<sup>1</sup> when tested by the paper chromatographic method and by the proposed revised colorimetric method, compared very closely with the reference PASNa used in this study.

## CONDITIONS AFFECTING THE COLORIMETRIC DETERMINATION OF MAP

**Effect of Time upon the Color Intensity Developed.**—As discussed by Seaman, *et al.* (3), the time after addition of the sulfuric acid and sodium nitrite is quite critical. As the result of our investigations, it was found that after the addition of the sodium carbonate solution the intensity of the color decreased rapidly over a 3-hour period. After 3 hours the color intensity stabilized (See Fig. 1.). For this reason the time lapse between the addition of the sodium carbonate solution and the measurement of color intensity has been changed to 3 hours in the proposed modified colorimetric method described under *Procedures* for MAP determinations in PAS and PASNa. This characteristic of the color development was found evident in all samples of PAS and PASNa tested.

**Effect of Change in Concentration of MAP upon the Absorbance Maximum of the Color Developed.**—To confirm the absorbance peak of 440  $m\mu$  for the MAP color intensity produced by the U.S.P. XVI method, absorption spectra in the 350 to 700  $m\mu$  range were obtained from two samples of PASNa known to contain 0.20% and less than 0.01% of MAP, using a Cary recording spectrophotometer

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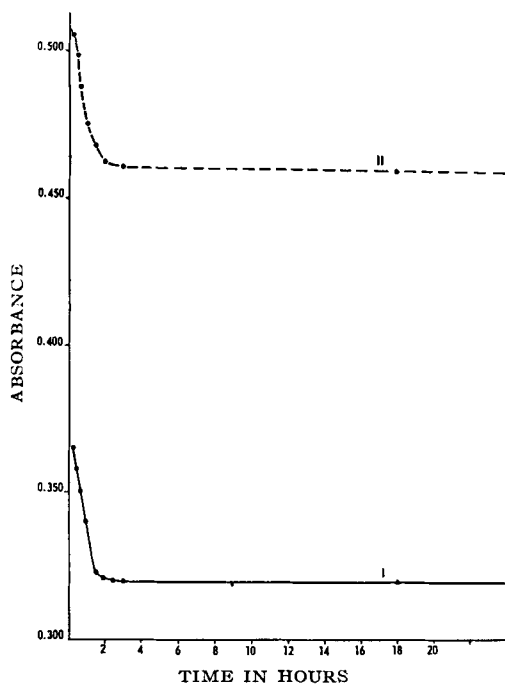


Fig. 1—Relationship of absorbance and time for PASNa samples. I, Reference quality PASNa containing less than 0.01% MAP; II, PASNa containing 0.12% MAP.

TABLE I.—EFFECT OF CHANGE IN CONCENTRATION OF MAP UPON ABSORBANCE MAXIMUM

Per cent of MAP in PASNa	Absorbance Maximum Found, $m\mu$	Comment on Curve Characteristics
0.02	420-425	Plateau occurs
0.04	420-425	Plateau occurs
0.08	425	Round curve occurs
0.10	425	Sharper peak occurs
0.15	430	Sharp peak occurs
0.20	430	Sharp peak occurs
0.30	430	Sharp peak occurs
0.50	435	Sharp peak occurs
1.00	435	Sharp peak occurs

(model 11). As indicated by Figure 2, the PASNa containing less than 0.01% of MAP showed a plateau-like peak in the 420-425  $m\mu$  range, and the PASNa containing 0.20% of MAP showed a peak at 430  $m\mu$ . As a result, a series of determinations were conducted, using solutions of PASNa containing known quantities of MAP ranging from 0.02 to 1.0%. The absorbance maxima obtained for these concentrations of MAP are shown in Table I. These results indicate a shift in the absorbance maximum with increasing MAP concentrations and justify the use of a 420 to 435  $m\mu$  range for the measurement of the maximum absorbance of the color developed by the proposed modified colorimetric method described under *Procedures for MAP in PAS and PASNa*.

Based upon our study of a number of production samples of PAS and PASNa and the correlation of data between the proposed modified colorimetric

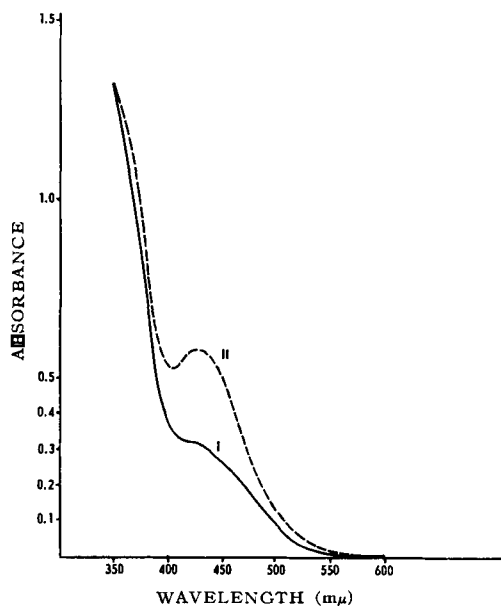


Fig. 2—Relationship of MAP concentration and absorbance maxima. I, Reference quality PASNa containing less than 0.01% MAP; II, PASNa containing 0.20% MAP.

method and paper chromatographic procedure (see Table II), the correction value for color produced other than by MAP should be 0.320 rather than 0.372. The absorbance values of our reference PASNa and the PASNa sample from Dr. Welsh also support the 0.320 value.

## PROCEDURES

**For PAS.**—Weigh accurately 500 mg. of PAS and transfer to a 100-ml. volumetric flask with the aid of 5 ml. of sodium hydroxide T.S. and 5 ml. of water. Mix to dissolve the sample and dilute the solution with water to about 80 ml. Add 10 ml. of dilute sulfuric acid (1 in 10), add water to volume, and mix. Within 150 seconds from the time the acid is added, transfer 5 ml. of this solution to a second 100-ml. volumetric flask immersed in an ice bath and containing 50 ml. of water at 0 to 5°, and add 2.5 ml. of sodium nitrite solution (1 in 100). Mix and allow to stand in the ice bath for 3 minutes  $\pm$  5 seconds. Add 25 ml. of sodium carbonate T.S., mix, and place the flask in a water bath at 25° for 15 minutes. Add water to volume, mix, and allow the solution to stand at 25° for 3 hours. Determine the absorbance of the solution at the absorbance maximum observed in a range of 420 to 435  $m\mu$  in a 1-cm. cell with a suitable spectrophotometer, using water as the blank. Calculate the per cent of MAP by the formula  $(A - 0.320)/0.84$ , in which A is the absorbance of the solution, 0.320 is the absorbance correction factor representing color produced other than by the reaction of MAP initially present, and 0.84 is the factor converting absorbance to per cent of MAP in PAS.

**For PASNa.**—Weigh accurately 690 mg. of PASNa and transfer to a 100-ml. volumetric flask. Add 80 ml. of water and 1.8 ml. of sodium hydroxide

TABLE II.—PER CENT OF MAP FOUND BY THE DIFFERENT METHODS TESTED

Sample of PASNa Tested	Per cent MAP Found		
	By Paper Chromatography	By U.S.P. XVI Method	By Proposed Modified Method
Reference Sample	Less than 0.01	Negligible <sup>a</sup>	Less than 0.01
Sample A	0.04	Negligible	0.03
B	0.12	0.08	0.12
C	0.01	Negligible	0.01
D	...	Negligible	0.07
E	...	Negligible	0.04
F	...	Negligible	0.04
G	...	Negligible	0.03
H	...	Negligible	0.04
FDA Sample	Less than 0.01	Negligible	Less than 0.01
Sample of PAS Tested			
Sample A	0.06	Negligible	0.05
B	1.25	1.17	1.21
C	0.65	0.57	0.65
D	0.02	Negligible	0.03
E	...	0.03	0.10
F	...	Negligible	0.07
G	...	Negligible	0.05
H	...	Negligible	0.06
J	...	Negligible	0.04
K	...	Negligible	0.08
L	...	Negligible	0.09
M	...	0.05	0.12
N	...	Negligible	0.03
P	...	0.04	0.11
Q	...	0.01	0.09

<sup>a</sup> The term negligible denotes that the absorbance value for the sample was less than 0.372, which by the U.S.P. XVI method would result in a minus percentage value for MAP content.

T.S., and mix. Add 10 ml. of dilute sulfuric acid (1 in 10), add water to volume, and mix. Within 150 seconds from the time the acid is added, transfer 5 ml. of this solution to a second 100-ml. volumetric flask immersed in an ice bath and containing 50 ml. of water at 0 to 5°, and add 2.5 ml. of sodium nitrite solution (1 in 100). Mix and allow to stand in the ice bath for 3 minutes  $\pm$  5 seconds. Add 25 ml. of sodium carbonate T.S., mix, and place the flask in a water bath at 25° for 15 minutes. Add water to volume, mix, and allow the solution to stand at 25° for 3 hours. Determine the absorbance of the solution at the absorbance maximum observed

in a range of 420 to 435  $m\mu$  in a 1-cm. cell with a suitable spectrophotometer, using water as the blank. Calculate the per cent of MAP by the formula  $(A - 0.320)/1.16$ , in which A is the absorbance of the solution, 0.320 is the absorbance correction factor representing color produced other than by the reaction of MAP initially present, and 1.16 is the factor converting absorbance to per cent of MAP in PASNa.

## SUMMARY AND CONCLUSIONS

1. The MAP content found by the proposed modified colorimetric method was comparable to that found by paper chromatography for the PAS and PASNa tested in this study.

2. The color produced by both the U.S.P. XVI method and the proposed modified colorimetric method stabilized after standing 3 hours at 25°.

3. The absorbance spectra obtained from the U.S.P. XVI method and the proposed method showed the absorbance maxima to be between 420 and 435  $m\mu$ .

4. The correction factor for color produced other than that by MAP should be changed from 0.372 to 0.320.

5. The values of 0.84 and 1.16 for conversion of absorbance to per cent MAP are correct.

6. The U.S.P. XVI procedure for the determination of MAP in PAS and PASNa is basically reliable.

7. It is recommended that the modifications, as herein described, be adopted by the U.S.P. XVI to correct minor sources of error in the determination of MAP in PAS and PASNa.

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