

Determination of *m*-Aminophenol (MAP) in Calcium Aminosalicylate [Ca(PAS)₂]

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The MAP test in the N.F. XI monograph for Ca(PAS)₂ has been re-evaluated. Experimental data showed the test to be in need of minor changes to correct sources of error. Based on this study, recommendations are made to use sulfuric acid instead of hydrochloric acid, to change the correction factor, to determine maximum absorbance through a range of 425 to 435 m μ , and to change the time allowed for color development to 3 hours.

THE MAP test in the N.F. XI monograph for Ca(PAS)₂ (1) is basically a good analytical method. However, our previous work with aminosalicyclic acid (PAS) and sodium aminosalicylate (PASNa) (2) showed that certain discrepancies existed in the MAP test. Subsequent studies made on Ca(PAS)₂ showed that the variables affecting the MAP test for PAS and PASNa applied also to the MAP test for Ca(PAS)₂.

To establish the absolute MAP content of some production samples of Ca(PAS)₂, the chromatographic procedure used in our previous studies of PAS and PASNa (2) was applied (see Table I).

EXPERIMENTAL

Proposed Modified Colorimetric Method for MAP in Ca(PAS)₂.—The proposed revisions of the N.F. XI test for MAP, as described under *Procedure*, were performed on samples of Ca(PAS)₂. The N.F. XI test for MAP was performed also on the same samples of Ca(PAS)₂. The basis for the revisions proposed are discussed under *Conditions Affecting the Colorimetric Determination of MAP*.

Conditions Affecting the Colorimetric Determination of MAP

Effect of Acid Used upon Absorbance Maximum of the Color Developed.—Samples of Ca(PAS)₂ tested by the N.F. XI method, using hydrochloric acid, gave absorbance values at 440 m μ that were about 3% lower than absorbance values obtained using sulfuric acid with the N.F. XI procedure (see Table II). In the proposed modified method described under *Procedure*, the absorbance values using hydrochloric acid were about 3 to 5% lower than were the absorbance values using sulfuric acid. Also, it was found that the use of hydrochloric acid in the initial solution sometimes produced a precipitate which could complicate the test. Based on these results and to be consistent with the proposed revised colorimetric procedure recommended for MAP in PAS and PASNa in our previous studies (2), sulfuric acid was selected.

Effect of Time upon the Color Intensity Developed.—As had been established by our previous investigations (2), the color intensity stabilized in about 3 hours after the addition of the sodium carbonate solution. Since our studies showed this to occur in the MAP test for Ca(PAS)₂ as well, 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 revised colorimetric method for MAP in Ca(PAS)₂, as described under *Procedure*. This characteristic in the color development was found evident in all samples of Ca(PAS)₂ tested.

Effect of Change in Concentration of MAP upon the Absorbance Maximum of the Color Developed.—As was shown in our previous studies (2), a shift in absorbance maximum occurred as the concentration of MAP in Ca(PAS)₂ was varied from 0.02 to 1.0% (see Table III). These results justified the use of a 425 to 435 m μ range for the measurement of absorbance of the color developed by the proposed revised colorimetric method described under *Procedure* for the determination of MAP in Ca(PAS)₂. None of the samples of Ca(PAS)₂ tested showed absorbance maxima at 440 m μ .

Absorbance Correction Value for Color Produced Other Than by MAP.—Based upon our study of a number of production samples of Ca(PAS)₂ and the correlation of data between the paper chromatographic procedure and the proposed modified colorimetric method for MAP, it was clearly established that the correction value for color produced other than by MAP should be 0.320 rather than 0.372 (see Table I).

Procedure

Weigh accurately an amount, calculated on the basis of the *Assay*, of Calcium Aminosalicylate equivalent to 562 mg. of anhydrous calcium aminosalicylate (500 mg. of aminosalicyclic acid), and transfer to a 100-ml. volumetric flask. Add 1.8 ml. of sodium hydroxide T.S., and dilute to about 80 ml. with water. 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

TABLE I.—PER CENT OF MAP FOUND IN Ca(PAS)₂ BY THE DIFFERENT METHODS TESTED

Sample of Ca(PAS) ₂ Tested	MAP Found, %		
	By Paper Chromatography	By N.F. XI Method	By Proposed Modified Method
I	<0.01	negligible ^a	<0.01
II	<0.01	negligible	<0.01
III	<0.01	negligible	<0.01
IV ^b	...	0.04	0.14
V ^b	...	0.05	0.17
VI ^b	0.11	negligible	0.11
VII	...	negligible	0.03
VIII	...	negligible	0.03
IX	<0.01	negligible	<0.01
X	...	negligible	<0.01
XI	...	negligible	0.01
XII	...	negligible	<0.01

^a The term "negligible" denotes that the absorbance value for the sample was <0.372, which by the N.F. XI method would result in a minus percentage value for MAP content.
^b Experimental laboratory samples stored for 8 years.

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TABLE II.—EFFECT OF ACID USED UPON THE ABSORBANCE MAXIMUM OF THE COLOR DEVELOPED

Sample	N.F. XI Method		Modified Method	
	Absorbance Using HCl	Absorbance Using H ₂ SO ₄	Absorbance Using HCl	Absorbance Using H ₂ SO ₄
I	0.315	0.324	0.312 (435 m μ)	0.321 (425 m μ)
II	0.310	0.320	0.305 (430 m μ)	0.320 (425 m μ)
III	0.305	0.315	0.307 (430 m μ)	0.322 (425 m μ)

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

MAP in Ca(PAS) ₂ , %	Absorbance Maximum Found, m μ	Comment on Curve Characteristics
0.02	425	Sloping plateau occurs
0.05	425	Plateau occurs
0.10	430	Round curve occurs
0.20	430-435	Round curve occurs
0.50	435	Sharp peak occurs
1.00	435	Sharp peak occurs

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, allow the solution to stand at 25° for 3 hours, and filter. Determine the absorbance of the clear solution at the absorbance maximum observed in a range of 425 to 435 m μ in a 1-cm. cell with a suitable spectrophotometer, using water as the blank. Calculate the per cent of *m*-aminophenol by the formula ($A - 0.320/1.09$), 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 *m*-aminophenol initially present, and 1.09 is the factor converting absorbance to per cent of *m*-aminophenol in Calcium Aminosalicylate.

SUMMARY AND CONCLUSIONS

1. The MAP content found by the proposed modified colorimetric method was comparable to that found by paper chromatography for the Ca(PAS)₂ tested in this study.
2. In the addition of acid to the initial solution in the MAP test, sulfuric acid should be added instead of hydrochloric acid.
3. The color produced by both the N.F. XI and the proposed modified colorimetric method stabilized after standing for 3 hours at 25°.
4. The absorbance spectra obtained for MAP by both the N.F. XI method and the proposed modified colorimetric method showed the absorbance maxima to be between 425 and 435 m μ .
5. The correction factor for color produced other than by MAP should be 0.320, not 0.372.
6. The N.F. XI method for the determination of MAP in Ca(PAS)₂ is a basically reliable procedure.
7. The value, 1.09, for the conversion of absorbance to per cent MAP was correct.
8. It is recommended that the modifications, as herein described, be adopted by N.F. XI to correct minor sources of error in the determination of MAP in Ca(PAS)₂.

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Ultraviolet Emission Spectrographic Determination of Trace Palladium to Ascertain the Method Used to Produce Tetracycline

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Ultraviolet emission spectrography may be readily employed to determine trace amounts of palladium in ashed samples of tetracycline. Palladium is not found in samples prepared by direct fermentation and is always found when chlortetracycline is dechlorinated using palladium catalytically.

TWO KNOWN commercial methods are used to produce the antibiotic tetracycline. The catalytic reduction of chlortetracycline using palladium or another of the noble metals (1) and the direct fermentation of the microorganism *Streptomyces aureofaciens* (2).

METHOD

The general procedure is essentially the carbon arc method of qualitative analysis by Harvey (3) and Nachtrieb (4). The sensitivity of the method was increased by using the slowly rotating slotted disk and stationary emulsion method (5). Using a 35-mg. charge, 0.1 mcg. or 3 p.p.m. can be detected. A charge containing 3.5 mcg. or 100 p.p.m. of palladium produces up to 10 spectral lines of the metal in the region 3500 to 2450 Å.

The 600-800° ash cake is ground fine in the porcelain crucible. The exposure is 3 min. as required by the 11-slot disk (5). The spectral line

palladium 3242.703 Å. is used for detecting the least amount of palladium, even though it is theoretically not the strongest. It is conveniently close to copper 3247.540 Å. for positive identification and has very little interference from a carbon line.

The length, width, and density of the line are mentally integrated into an order of magnitude value (3, 4) and applied to the least detectable concentration of 3 p.p.m. The least detectable concentration is different in each ashed sample. It depends greatly upon the amount of reduction in weight by dry ashing at 600 to 800°.

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