UV Spectrophotometric Analysis of Aminobenzoic Acid Tablets

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Abstract □ An accurate UV spectrophotometric procedure was developed for the assay of aminobenzoic acid tablets. The crushed tablet was treated with 95% ethanol, and this alcoholic solution was diluted with distilled water. The resulting solution was assayed by absorption spectroscopy. The reproducibility and short-time requirement of the assay suggest that such a UV spectrophotometric method should be considered as the official assay method for *p*-aminobenzoic acid samples.

Keyphrases □ Aminobenzoic acid tablets—UV spectrophotometric analysis □ UV spectrophotometry—analysis, aminobenzoic acid tablets

An accurate analysis procedure for any pharmaceutical preparation is obviously a prerequisite before the preparation can be marketed. From the standpoint of industrial application, it is desirable that the analytical procedure be rapid and easily automated.

It was decided to investigate the development of such an analytical procedure for aminobenzoic acid tablets for several reasons. First, the USP XVIII (1) assay procedure for *p*-aminobenzoic acid is time consuming because it is based on the titration of a *p*-aminobenzoic acid–hydrochloric acid–ice solution with 0.1 *M* sodium nitrite until a blue color is produced immediately when a glass rod dipped in the solution is touched to starch iodide paper. This procedure is subject to variations between individuals in terms of deciding when the endpoint is reached.

Aminobenzoic acid tablets are sold over-the-counter as a B-complex factor in a number of health food stores. Based on the growing popularity of the health food diet, it was felt that the development of a faster assay procedure and the analysis of several aminobenzoic acid tablets selected at random would yield valuable information concerning the potency of the health food aminobenzoic acid formulation.

In addition to the official USP XVIII titration method for *p*-aminobenzoic acid analysis, numerous other titration procedures have been reported (2–8). Other reported analytical procedures include reflectance spectrometry (9), the Bratton–Marshall method (10), diazotization followed by coupling to acid (11), photocolorimetry (12), microbiological methods (13), and coprecipitation by zinc ferrocyanide of procaine in a procaine–*p*-aminobenzoic acid mixture (14). Analytically useful fluorescence and phosphorescence signals have also been obtained for *p*-aminobenzoic acid (15).

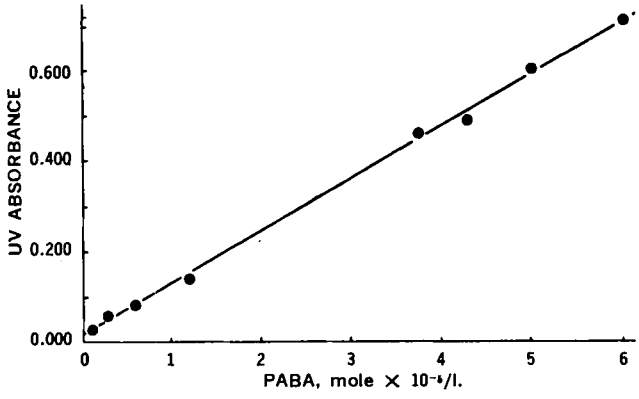

Figure 1—*Plot of UV absorbance* versus p-*aminobenzoic acid* (*PABA*) *concentration.*

EXPERIMENTAL

Materials—*p*-Aminobenzoic acid[1] was used without further purification in preparing the standard solutions for the analytical curve. Two bottles of 100-mg. aminobenzoic acid tablets[2] were purchased from a local health food store. For the UV measurements, a grating spectrophotometer[3] was used.

Analytical Curve—Five samples of *p*-aminobenzoic acid with weights of 90.05, 95.00, 100.14, 105.05, and 110.03 mg. were weighed, and each sample was treated in the following manner. The sample was placed in a 50-ml. volumetric flask, and 50 ml. of 95% ethanol was added. The solution was shaken and placed in an ultrasonic cleaner to ensure complete dissolution of the *p*-aminobenzoic acid.

Using a Lambda pipet, 0.1 ml. of the alcoholic stock solution was added to 50 ml. of distilled water in a volumetric flask. Four such solutions were prepared, and the absorbance at 268 nm. of each solution at ambient temperature was then recorded. The absorbance was plotted against the milligrams *p*-aminobenzoic acid in the original alcoholic stock solution.

***p*-Aminobenzoic Acid Isolation from Tablets**—Each tablet was wrapped inside glassine weighing paper and then crushed. The powder was transferred to a 50-ml. volumetric flask, and 50 ml. of 95% ethanol was added. The solution was shaken, placed in the ultrasonic cleaner, and then filtered. Five 0.1-ml. aliquots were taken and each was added to 50 ml. of distilled water. The absorbance at 268 nm. was measured for each solution.

RESULTS AND DISCUSSION

To determine the limit of detection and to check Beer's law for the UV analysis, a series of *p*-aminobenzoic acid solutions, ranging from 1.2×10^{-6} to 6.04×10^{-5} *M*, was prepared. Figure 1 is a plot of absorbance *versus* *p*-aminobenzoic acid concentration. The UV plot is linear over the range considered. The limit of detection for UV analysis is approximately 1.2×10^{-6} *M*. This is equivalent to a tablet containing 4.14 mg. of *p*-aminobenzoic acid.

Table I summarizes the data for the absorbance values of the analysis of 90–110 mg. *p*-aminobenzoic acid. This is in the range of the commercial tablets tested.

[1] Fisher Lot No. 280288.
[2] Distributed by Radiance Products Co., Alhambra, Calif. (Lot Nos. 1337809 and 1347610).
[3] Beckman BD-GT.

Table I—UV Absorbance Data for Various Concentrations of *p*-Aminobenzoic Acid

Solution	*p*-Aminobenzoic Acid Initially Weighed, mg.	*p*-Aminobenzoic Acid Concentration in the Analyzed Solution, *M*	UV Absorbance (268 nm.)	$X \pm \sigma$
A1	90.05	2.61×10^{-5}	0.326	0.328 ± 0.002
A2			0.371[a]	
A3			0.328	
A4			0.329	
B1	95.00	2.75×10^{-5}	0.359	0.350 ± 0.006
B2			0.348	
B3			0.350	
B4			0.345	
C1	100.14	2.91×10^{-5}	0.378	0.366 ± 0.011
C2			0.360	
C3			0.359	
C4			0.410[a]	
D1	105.05	3.05×10^{-5}	0.373	0.377 ± 0.005
D2			0.376	
D3			0.382	
D4			0.430[a]	
E1	110.3	3.19×10^{-5}	0.400	0.400 ± 0.006
E2			0.399	
E3			0.393	
E4			0.407	

[a] Value excluded from data.

Table II—UV Absorbance Readings and Milligrams *p*-Aminobenzoic Acid for the Two Lots of Aminobenzoic Acid Tablets Tested

Lot	Tablet	Average Absorbance ± σ[a]	Average Milligrams *p*-Aminobenzoic Acid ± σ[a]	Average Milligrams *p*-Aminobenzoic Acid/Tablet/Lot
1337809	1	0.394 ± 0.011	108.67 ± 3.20	103.40 ± 4.90
	2	0.379 ± 0.014	104.26 ± 4.02	
	3	0.383 ± 0.011	105.38 ± 3.22	
	4[b]	0.365 ± 0.012[b]	100.14 ± 3.46[b]	
	5	0.357 ± 0.007	97.93 ± 1.98	
1347610	1	0.372 ± 0.009	102.15 ± 2.71	104.22 ± 4.47
	2	0.372 ± 0.009	102.21 ± 2.60	
	3	0.388 ± 0.015	106.84 ± 4.31	
	4	0.395 ± 0.007	108.84 ± 2.29	
	5[b]	0.365 ± 0.016[b]	100.29 ± 4.59[b]	

[a] Average of five determinations. [b] Average of four determinations.

Five tablets from each of the two lots were analyzed, and five absorbance determinations were made on each tablet. These data are summarized in Table II. The tablets from Lot No. 1337809 had an average value of 103.40 ± 4.90 mg. *p*-aminobenzoic acid per tablet, while those from Lot No. 1347610 had an average value of 104.22 ± 4.47 mg. *p*-aminobenzoic acid per tablet.

Statistical analysis of the data in Fig. 1 gave the following linear regression equation:

$$Y = 0.114874X + 0.0136 \qquad \text{(Eq. 1)}$$

The linear correlation coefficient is 0.999. The statistical analyses were performed on a calculator[4] using standard programs. Statistical analysis of the regression data in Table I (UV absorbance *versus* milligrams *p*-aminobenzoic acid initially weighed) gave the following linear regression equation:

$$Y = 0.003408X + 0.023469 \qquad \text{(Eq. 2)}$$

with a linear correlation coefficient of 0.971.

Based on the fact that this is a completely randomized experiment with equal sample sizes, the statistical tests based on a nested classification of the hypotheses that the *p*-aminobenzoic acid content in the two lots and among the individual tablets is equal is shown in Table III. The ANOVA values were obtained from the five *p*-aminobenzoic acid milligram values for each of the 10 tablets.

[4] Monroe Epic 3000.

Table III—ANOVA for the Assayed Weight of *p*-Aminobenzoic Acid

Source	*df*	*SS*	*MS*	*F*, calc.
Lots	1	0.0000245	0.0000245	0.02
Tablets/lot	8	0.0083615	0.0010452	4.64
Determinations/ tablet	40	0.0090040	0.0002251	—
Total	49	114.24	—	—

To test the hypothesis of equal *p*-aminobenzoic acid content between the lots, $F = 0.02$. From the *F*-table (16), $F^{1}_{8(0.05)} = 5.32$. Therefore, the hypothesis should be accepted. To test the hypothesis of equal *p*-aminobenzoic acid content among the tablets, $F = 4.64$. From the *F*-table (16), $F^{8}_{40(0.05)} = 2.18$. Therefore, the hypothesis should be rejected.

The statistical analysis of the data shows that there is no significant variation between the two lots tested but that the variation among the tablets is significant.

These results show that the UV method of analysis is applicable to aminobenzoic acid tablet analysis. This UV method is fast and accurate, and it offers a substantial improvement over the USP XVIII titration procedure.

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Dimethylformamide Dimethylacetal as a Derivatizing Agent for GLC of Barbiturates and Related Compounds

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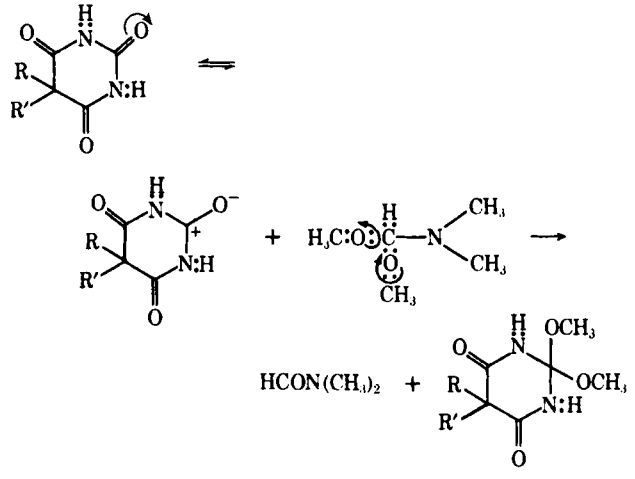
Abstract □ Dimethylformamide dimethylacetal reacted quantitatively and reproducibly with glutethimide, phenobarbital, hexobarbital, secobarbital, amobarbital, aprobarbital, and pentobarbital to form the corresponding acetals. The acetal derivative could be chromatographed easily on a 3% OV-17 column using either isothermal or programmed conditions (for multiple separations), permitting the separation and determination of the compounds studied with good precision and speed. NMR evidence and mass fragmentography was used to confirm the structure of the derivative. The glutethimide derivative was susceptible to solvent-induced reversibility.

Keyphrases □ Dimethylformamide dimethylacetal—used as derivatizing agent for GLC of barbiturates, glutethimide □ Barbiturates—GLC analysis, dimethylformamide dimethylacetal as derivatizing agent □ Glutethimide—GLC analysis, dimethylformamide dimethylacetal as derivatizing agent □ GLC—analysis, barbiturates and glutethimide, dimethylformamide dimethylacetal as derivatizing agent

The vast use of the 5,5-disubstituted barbituric acid derivatives, glutethimide, and methyprylon as sedatives as well as the interest they have commanded in forensic science has led to numerous reports on the use of GLC as an analytical method for their determination (1–5). The most recent literature (1–3) relied on the formation of their *N*-methyl derivatives prior to GLC (2) or on *in situ* formation in the chromatograph injector port (1–3). The derivative was formed by the action of trimethylanilinium hydroxide, a reagent requiring a time-consuming synthesis[1] (3). While the formation of the methylated compounds removed the disadvantages of adsorption, tailing, and column contamination previously experienced with GLC of the parent barbiturates, most of the columns and conditions used did not remove the failure of baseline separation when chromatographing mixtures.



Scheme I

The inadequacies prompted this laboratory to investigate other derivative routes, which led to a study of the chromatographic behavior of the product of barbiturates with dimethylformamide dimethylacetal (I)[2].

Depending on the reacting compounds and conditions employed, dimethylformamide–dialkylacetals react to form either acetals with imides (6) or formamidine derivatives with amides (7). Since I decomposes during the reaction to form both CH_3^+ and OCH_3^-, either *N*-methylation or acetal formation with barbiturates was seemingly possible. The path taken would depend only on the relative ease of proton abstraction from the subject compound, as opposed to carbonyl polarization.

[1] After completion of this work, Pierce Chemical Co. began marketing the reagent as MethElute.
[2] Aldrich Chemical Corp., Milwaukee, Wis.