Spectrophotometric Analysis of Phenobarbital and Pentobarbital in Pharmaceuticals

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An analytical procedure has been developed for measuring the concentration of phenobarbital and pentobarbital in mixtures of these substances, *e.g.*, in pharmaceuticals. This new method is a spectrophotometric procedure based upon the shift in ultraviolet absorbance of pentobarbital subsequent to dealkylation in sulfuric acid.

IN THE COURSE of their investigation of the structure of the metabolites of pentobarbital, Maynert and Washburn (1) found that certain dialkyl barbituric acids are subject to dealkylation in sulfuric acid. These workers reported that 5,5-dialkyl barbituric acids containing a secondary group lost this group in sulfuric acid. For example, 5-ethyl-5-(1-methylbutyl)-barbituric acid was dealkylated to 5-ethyl barbituric acid. Monoalkyl derivatives, such as 5-(1-methylbutyl)-barbituric acid, were found to be stable in this menstruum, as were 5,5-disubstituted barbituric acids containing two primary alkyl groups or a phenyl and a primary alkyl group.

Brooker (2) found this reaction mechanism could be utilized in the analysis of mixed barbiturates by paper chromatography. He demonstrated that the chromatographic resolution of amylobarbital and pentobarbital could be markedly improved by selectively converting the pentobarbital to 5-ethyl barbituric acid with hot sulfuric acid.

Curry (3) reported that the ultraviolet absorbance maximum of pentobarbital shifts from 240 to 268 millimicrons as a consequence of dealkylation. He found that phenobarbital under these conditions was completely destroyed and the ultraviolet absorbance of this substance became zero as a consequence of this hot acid treatment.

Work in this laboratory has confirmed the shift in the ultraviolet absorbance maximum of pentobarbital. However, we found that the absorbance of phenobarbital is essentially unchanged by hot sulfuric acid treatment (see Fig. 1). These results are consistent with Maynert and Washburn's observations concerning the stability of 5,5-disubstituted barbituric acids in sulfuric acid solution

These data suggested the possibility of using this technique for measuring the concentration of both phenobarbital and pentobarbital in pharmaceuticals containing both of these substances.

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EXPERIMENTAL

In order to define the necessary conditions for the proposed analytical technique, the following experiments were performed:

Effect of Heating Time On Dealkylation of Pentobarbital.—An aqueous solution was prepared by dissolving 25.0 mg. of pentobarbital in 5.0 ml. of 0.13 *N* sodium hydroxide. One-milliliter aliquots were mixed with 9.0 ml. of concentrated sulfuric acid and heated at 100° for increments of time. Subsequent to cooling, these samples were diluted to 50.0 ml. with distilled water. Five-milliliter aliquots were mixed with 5.00 ml. of 22.5% ammonium hydroxide and then diluted to 100.0 ml. with distilled water. The absorbance of these solutions at 240 and 268 mμ was then measured, using a Beckman DU spectrophotometer. The results are recorded in Table I. It would seem from these data that timing of the acid treatment step is not critical. Thirty minutes at 100° was used for all subsequent work.

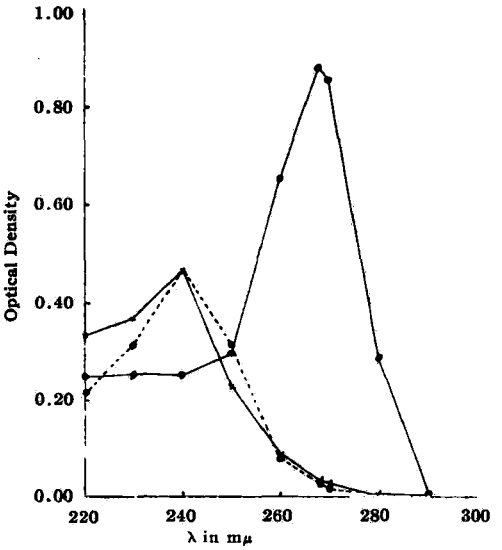

Fig. 1.—U.V. absorbance of phenobarbital and pentobarbital before and after treatment with hot sulfuric acid: ⊙-⊙-⊙ 10 mcg. pentobarbital/ml. before hot sulfuric acid treatment; ⊙—⊙—⊙ 10 mcg. pentobarbital/1 ml. after hot sulfuric acid treatment; ×—×—× 10 mcg. phenobarbital/ml. before and after hot sulfuric acid treatment.

TABLE I.—EFFECT OF HEATING TIME ON PENTOBARBITAL DEALKYLATION

Time of Heating, min.	Absorbance 240mμ	Absorbance 268mμ
0	0.466	0.022
15	0.269	0.846
30	0.252	0.880
60	0.252	0.880

Effect of Sulfuric Acid Concentration on Rate of Dealkylation of Pentobarbital.—An aqueous solution was prepared by dissolving 25.0 mg. of pentobarbital in 5.0 ml. of 0.13 *N* sodium hydroxide. One-milliliter aliquots were mixed with 9.0 ml. of sulfuric acid of varying strengths. Subsequent to cooling, these samples were diluted to 50.0 ml. with distilled water. Five-milliliter aliquots were mixed with 5.0 ml. of 22.5% ammonium hydroxide and then diluted to 100.0 ml. with distilled water. The absorbances were then measured at 240 and 268 mμ. The results are recorded in Table II.

TABLE II.—EFFECT OF ACID CONCENTRATION ON PENTOBARBITAL DEALKYLATION

Concentration of Acid Added	Absorbance 240mμ	Absorbance 268mμ
21 *N*	0.445	0.018
30 *N*	0.431	0.102
36 *N*	0.252	0.880

It is apparent from these data that acid concentration is critical and must be carefully controlled. In all of the subsequent work, 36 *N* sulfuric acid was used in the analysis.

Interfering Substances.—Only those substances present in the pharmaceutical preparation of interest in this laboratory were evaluated with respect to their effect on the proposed analytical technique. It was found that preliminary purification by filtration and solvent extraction eliminated most of these substances prior to acid treatment. Thus, lactose, calcium stearate, procaine hydrochloride, and N-ethyl-3-piperidyl benzilate methobromide were found to be without significant effect on the proposed barbiturate analysis.

Effect of pH on Ultraviolet Absorbance of Phenobarbital, Pentobarbital, and Ethyl Barbituric Acid.—It is known that pH has significant effect on ultraviolet absorbance of barbiturates and their derivatives. However, it is extremely simple to prepare samples of exactly reproducible pH by mixing measured volumes of reagents of known concentration. Our experience indicated that if ordinary quantitative techniques are observed in measuring the standardized reagents employed, the variation in absorbance on a given sample will be negligible.

Summation of Experimental Procedure—Ten milliliters of an ethereal extract containing 2.5–5.0 mg. each of phenobarbital and pentobarbital was transferred to a 2.2 × 24 cm. test tube. The ether was carefully evaporated off with the aid of a warm water bath. The residual barbiturates were then dissolved in 1.0 ml. of 0.13 *N* sodium hydroxide and mixed with 9.0 ml. of 36 *N* sulfuric acid. This mixture was heated at 100° for 30 minutes and then cooled to RT. The sample was then placed in an ice bath and diluted to 50.0 ml. with distilled water. Five milliliters of this solution was mixed with 5.0 ml. of 22.5% ammonium hydroxide and then diluted to 100.0 ml. with distilled water. The absorbance was then measured against a reagent blank at 240 and 268 mμ.

TABLE III.—EFFECT OF HOT SULFURIC ACID TREATMENT UPON ULTRAVIOLET ABSORBANCE OF PHENOBARBITAL AND PENTOBARBITAL

Wavelength, mμ	Absorbance of Phenobarbital[a] Heated	Absorbance of Phenobarbital[a] Unheated	Absorbance of Pentobarbital[a] Heated	Absorbance of Pentobarbital[a] Unheated
220	0.336	0.336	0.244	0.216
230	0.366	0.366	0.254	0.309
240	0.465	0.466	0.252	0.466
250	0.220	0.222	0.296	0.308
260	0.082	0.083	0.656	0.078
268	0.032	0.031	0.880	0.022
270	0.022	0.021	0.856	0.015
280	0.003	0.003	0.282	0.001
290	0.000	0.000	0.006	0.000

[a] Concentration of each barbiturate is 10.0 mcg./ml.

Pure samples of phenobarbital and pentobarbital were analyzed by this technique and the absorbances were measured at intervals throughout the ultraviolet spectrum. Similar samples were prepared and the absorbances similarly measured after addition of the acid but prior to heat treatment. These data are recorded in Table III. These data were then used to calculate the concentration in unknown sample mixtures as follows: Let X = mcg. of pentobarbital/ml., and Y = mcg. of phenobarbital/ml. Then, $0.465\ Y/10 + 0.252\ X/10$ = absorbance at 240 mμ and $0.032\ Y/10 + 0.880\ X/10$ = absorbance at 268 mμ.

Substituting in the absorbance values observed on a given sample and then solving simultaneously, one can determine the concentration of each of the barbiturates in the dilute ammoniacal solution. Back calculating by substituting in the appropriate dilution factors, one can determine the concentration of each of the barbiturates in the original sample.

RECOVERY EXPERIMENT

Mixtures were prepared containing varying proportions of phenobarbital and pentobarbital. Aliquots of the resultant solutions were analyzed in the proposed manner. The results obtained are recorded in Table IV.

TABLE IV.—PER CENT RECOVERY OF PHENOBARBITAL AND PENTOBARBITAL IN KNOWN MIXTURES

Sample	Phenobarbital, mcg./ml. Theor.	Found	% Recovery	Pentobarbital, mcg./ml. Theor.	Found	% Recovery
1	6.04	6.01	99.7	4.02	4.08	101.3
2	5.04	5.04	100.0	5.02	5.04	100.1
3	4.03	4.09	101.4	6.02	6.11	101.3

REFERENCES

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