SUMMARY

Several physical properties have been studied for the systems of camphor with phenol and the three cresols, respectively. These include freezing curves, densities, partial solubilities in water, partial vapor pressures, temperature effects on mixing liquid solutions, and cryoscopic molecular weight determinations. Melting points for equimolecular complexes were found to be $-12°$ C. for camphor-phenol and $-18°$ C. for camphor-*o*-cresol. Complexes with the other two cresols could not be crystallized because of the high viscosity of the solutions. A convenient method of measuring partial pressures and a method of interpretation of temperature effects on mixing were devised.

Evidence derived primarily from the latter two properties, but supported also by the others, points to the existence of at least two complexes in each mixture, namely, those of one mole of camphor with one and two moles, respectively, of phenol or cresol. The former predominates in the case of phenol, but the latter in the case of all three cresols. All these complexes are partly dissociated in solution and rapidly decomposed on chemical analysis. The concentration of free cresol in an equimolecular mixture with camphor is 6% to 9%, and in a 20 weight per cent mixture with camphor the free *m*-cresol is about $1^1/_2\%$.

Although this investigation has been confined to the physicochemical properties of phenol-camphor and cresol-camphor mixtures, it is interesting to speculate as to the probable bearing of these properties on the known pharmacological properties of such mixtures. The low content of free phenol or cresol found probably accounts for the high toleration which wounds and tissue show to these mixtures. The fact that these are equilibrium mixtures means that they will liberate free phenol or cresol as fast as that originally present is consumed and this may explain their known antiseptic and surface anesthetic effects.

REFERENCES

(1) Günther, P., and Peiser, M., *Z. physik. Chem.*, 128 (1927), 189–202.

(2) See Lajoux, H., *Chem. Abstr.*, 11 (1917), 3376[8].

(3) Wood, J. K., and Scott, J. D., *J. Chem. Soc.*, 97 (1910), 1573.

(4) Kremann, R., Wischo, F., and Paul, R., *Monatsh.*, 36 (1915), 911.

(5) Leger, E., *Compt. rend.*, 111 (1890), 109–111.

(6) Fox, J. J., and Barker, M. F., *J. Soc. Chem. Ind.*, 37 (1918), 270T.

(7) Francis, A. W., and Hill, A. J., *J. Am. Chem. Soc.*, 46 (1924), 2498.

(8) Le Fevre, R. J. W., and Tidemann, C. G., *J. Chem. Soc.*, (1931), 1731.

(9) International Critical Tables, Vol. III, pages 29, 33.

(10) *Ibid.*, page 428.

(11) *Ibid.*, pages 208, 221, 223, 226.

A Method for the Quantitative Determination of Theobromine or Theobromine Salts and Phenobarbital in Mixtures

*By C. W. Bell**

Due to the introduction of many new organic drugs into medicine and the continued practice of combining two or more such drugs in a single dosage form, the analysis of medicinal preparations is a problem of increasing complexity. Occasionally as many as four active ingredients may be combined in a single dosage form and it is a very common practice to combine two organic compounds in a single dosage form. In such mixtures it is necessary to have a method for the separation and quantitative determination of each of the active constituents. Much progress has been made in this branch of pharmaceutical chemistry in the past few years but a critical survey of the literature revealed that a method for the quantitative determination of theobromine, or its salts, and phenobarbital in mixtures has not as yet been reported. In attempting to develop such a method the author was primarily concerned with the analysis of tablets containing phenobarbital and Theo-

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calcin. (Theocalcin is the proprietary name for the basic calcium salicylate of theobromine, a chemical compound consisting of one mol. each of calcium, salicylic acid and theobromine. Theoretically it contains 48.15% of theobromine.) In addition to Theocalcin-phenobarbital mixtures, the method has also been successfully applied to mixtures of theobromine and phenobarbital as will be brought out later.

LITERATURE SURVEY

Although the literature does not describe a method for the determination of theobromine and phenobarbital in mixtures, there are various methods reported for the determination of the two compounds individually. These various methods will be described briefly below under the headings of "Theobromine" and "Phenobarbital" but most of the methods reported for the determination of theobromine may be also applied to the various salts and compounds of theobromine, such as Diuretin and Theocalcin.

Phenobarbital.—In most simple preparations containing only phenobarbital, the phenobarbital is extracted by the use of ether or some other suitable immiscible solvent, the solvent evaporated and the amount of extracted phenobarbital determined by weight. An example of this method is the determination of phenobarbital in the Tablets of Phenobarbital N. F. (1). In addition to the above gravimetric method there are other types of gravimetric methods depending upon the formation of complex addition products such as the complex product formed by the reaction of phenobarbital with mercuric iodide as reported by Montignie (2); the complex salts formed with iodine in an alkaline solution as reported by Bougault and Guillou (3); complex mercury salts as reported by Fleury (4), and the copper pyridinyl phenobarbital formed by the use of Zwikker's reagent (5). Most of the methods depending upon the formation of complex additional salts are unreliable due probably to the inconsistent or unknown composition of the addition product.

The accuracy of the cobalt colorimetric method proposed by Dille and Koppanyi (6) is limited to about 6%, and the method also gives a positive reaction with theobromine.

In addition to the various gravimetric and colorimetric methods for estimating phenobarbital there have been several volumetric methods proposed which give very good results when applied to phenobarbital alone. Among the most important of these methods is the acidimetric method of Babick (7); the turbidimetric method of Budde (8), and Budde's turbidimetric method as modified by Kalinowski (9). The two last-named methods are based on the fact that silver nitrate reacts with phenobarbital in sodium hydroxide solution to form insoluble silver phenobarbital which is extremely weakly disassociated so that the remainder of the sodium hydroxide solution causes a separation of the silver oxide only gradually and incompletely.

Theobromine.—Like phenobarbital, theobromine may be determined in its simple preparations and compounds by the gravimetric method; that is, by either extracting or precipitating the alkaloid and weighing the extract or precipitate as theobromine. An example of this general method is the U. S. P. XI method for the determination of theobromine in Theobromine with Sodium Salicylate (10). In this general method a correction factor must be used because of the solubility of the theobromine. The various gravimetric methods usually give inconsistent results in the hands of different operators probably because of the slight variations in technique; therefore, various correction factors have been proposed.

For many years the most reliable volumetric method for the determination of theobromine was the iodometric method of Emery and Spencer (11). This method depends upon the precipitation of theobromine tetraiodide by the use of an excess of iodine and the determination of the excess of iodine by the use of a standard solution of thiosulfate. The method is more or less unreliable since it gives inconsistent results from one day to another and in the hands of different operators. To overcome these difficulties there

have been various modifications of the method of Emery and Spencer (12, 13, 14, 15, 16). These various modifications depend upon the same principle but vary the method and conditions of the precipitation of the tetraiodide and none of the modifications are very superior to the original method.

The author has found that the most reliable method for the determination of theobromine is the method of Boie (17). This method is much less time-consuming and consistently more accurate than the method of Emery and Spencer or its various modifications. This fact has been confirmed by several reports in the literature including Jorgensen (18) and Van Giffen (19) together with the fact that it has been recently approved as one of the tentative methods of the A. O. A. C. (20).

Briefly this method is based on the fact that silver nitrate reacts with theobromine to form silver theobromine and liberating nitric acid according to the following reaction:

$$C_7H_8N_4O_2 + AgNO_3 \rightarrow AgC_7H_7N_4O_2 + HNO_3$$

The nitric acid thus liberated is titrated with standard alkali. This method for the determination of theobromine has been successfully applied not only to theobromine alkaloid but to many mixtures and salts of theobromine.

EXPERIMENTAL

The usual procedures for the determination of two or more organic drugs of this nature involve the separation of the two or more components of the mixture by the use of a suitable immiscible solvent or mixture of such solvents, evaporating the solvent and determining the amount of extracted material by weight. In combinations of the Theocalcin or theobromine and phenobarbital type, this general procedure is more or less impractical, especially for the determination of phenobarbital, because of the small amount of phenobarbital in relation to the other components. The combinations on the market usually contain 5 grains of theobromine or a corresponding larger amount of a theobromine salt with either $^1/_4$ or $^1/_2$ grain of phenobarbital. Again, this general procedure could not be applied to Theocalcin-phenobarbital mixtures since the phenobarbital must be extracted from an acid solution and any solvent that might be selected to extract the phenobarbital from an acid solution of the mixture would also extract the liberated salicylic acid. Therefore, some other method had to be resorted to.

Experiments proved that salicylic acid does not interfere with the turbidimetric methods of Budde (8) and Kalinowski (9) for the determination of phenobarbital, so it was thought that perhaps this method or some modification of it might serve as a basis for the determination of phenobarbital in mixtures with theobromine calcium salicylate.

After reviewing the various methods outlined above for the determination of theobromine in various mixtures and compounds it was thought that perhaps Boie's acidimetric method (17) offered the best possibilities for adaptation to mixtures of the Theocalcin-phenobarbital type if the active components of the mixture could be separated. For this purpose, as can be seen from Table I, ether offers the best possibilities since phenobarbital is more soluble and theobromine less soluble in ether than in any one of the other solvents or combinations of these solvents investigated. The use of the alcohol-chloroform-ether mixture used in the N. F. method for extracting phenobarbital in various preparations is impractical because of the relatively greater solubility of theobromine. Although ether will extract a very slight amount of the theobromine along with the phenobarbital, the amount is insignificant and does not interfere with the methods outlined below. The ether, however, should be free from alcohol—this is very important.

Table I.—The Solubility of Theobromine and Phenobarbital in Seven Solvents (Compiled from Various Sources)

Note: Solubility given in Gm. per 100 cc. of Solvent at Room Temperature.

Solvent	Theobromine	Phenobarbital
Alcohol	0.040	12.500
Benzene	0.005	0.143
Carbon tetrachloride	0.020	Very slightly sol.
Chloroform	0.060	2.500
Ether	0.003	7.692
Petroleum ether	0.000	0.005
Water	0.050	0.100

METHODS

I. Mixtures Containing Phenobarbital and a Theobromine Salt Such as Theocalcin.—After numerous trials the following method for the analysis of Theocalcin-phenobarbital combinations was adopted:

Theobromine.—Weigh a sample of the mixture corresponding to approximately 15 grains of Theocalcin and transfer the sample to a 250-cc. beaker. Add 50 cc. of water followed by about 50 cc. of approximately 0.1*N* sulfuric acid solution. Boil slowly until a solution results or, if tablet diluents are present, until a homogeneous opalescent mixture results—about two or three minutes. Cool the solution to about 40° C. and then transfer it to a 250-cc. separatory funnel, rinsing the beaker with several small portions of boiling water. To the luke-

warm solution and washings in the separatory funnel add 50 cc. of ether, which is free from alcohol, and shake vigorously. Collect the lower or aqueous layer in another 250-cc. separatory funnel and filter the ether layer through filter paper, discarding the filtrate. Continue to shake out the aqueous solution and washings with three successive portions of 25 cc. of alcohol-free ether, shaking at least two minutes after each addition and each time collecting the aqueous layer in the other separatory funnel and filtering the ether layer. Finally collect the aqueous solution in a 500-cc. wide-mouth Erlenmeyer flask washing each of the two separatory funnels with several small portions of boiling water to remove any adhering material which might be present. Collect the washings in the Erlenmeyer flask and finally place the filter paper from the ether washings into the Erlenmeyer flask along with the extracted aqueous solution and washings. Boil the solution on a hot-plate until the ether has been removed from the solution and then cool to about 40° C. Add $1^1/_2$ cc. of Phenol Red solution (0.02%) and make the solution distinctly alkaline with a slight excess (1–2 cc.) of 0.1*N* sodium hydroxide solution. Then adjust the solution by the careful addition, drop by drop, of 0.1*N* sulfuric acid solution until the bluish red color is discharged and the first distinctly yellow color is produced. Then add about 40 cc. of approximately 0.1*N* silver nitrate solution and titrate the liberated nitric acid slowly with 0.1*N* sodium hydroxide solution until the solution just acquires a bluish red color. Each cc. of 0.1*N* sodium hydroxide solution consumed in the neutralization of the nitric acid (that is, after the addition of the silver nitrate solution) is equivalent to 0.01801 Gm. of theobromine.

Phenobarbital.—Accurately weigh a sample of the mixture corresponding to about four grains of phenobarbital. Transfer the sample to a 250-cc. beaker and add 150 cc. of 2*N* sulfuric acid. Boil slowly until the sample is in solution, or if tablet diluents are present, until a homogeneous, opalescent mixture results, usually about three minutes. Cool the mixture to about 40° C. and transfer it to a 400-cc. separatory funnel, rinsing the beaker with several small portions of boiling water. To the lukewarm solution and washings in the separatory funnel add 50 cc. of alcohol-free ether and shake for about three minutes. Collect the ether layer in a 250-cc. Erlenmeyer flask, filtering through a pledget of cotton. Then continue to shake out the aqueous solution and washings with four successive portions of 25 cc. of alcohol-free ether, shaking at least two minutes after each addition; each time the ether layer is filtered through the pledget of cotton and collected in the Erlenmeyer flask. Two separatory funnels are used alternately as in the case with the theobromine determination. Finally wash the stems of the separatory funnels with a little ether and wash the cotton and funnel with several small portions of ether, collecting the washings in the Erlenmeyer flask. The aqueous layer is discarded and the combined ether extract and washings are heated on a water bath to distil off the solvent. The last few cc.'s of ether are allowed to evaporate at room temperature or not over 40° C. The residue is dissolved in exactly 100 cc. of a solvent composed of 50 cc. of ethyl alcohol, 10 cc. of water and 40 cc. of 1.0*N* sodium hydroxide solution. (The components of this solvent must be mixed and cooled to room temperature before adding to the phenobarbital extract.) After the phenobarbital is dissolved, the solution is filtered through a quantitative grade of filter paper and the first ten cc. of the filtrate are discarded. Transfer exactly 75 cc. of the filtrate, which must be perfectly clear, to a 250-cc. Erlenmeyer flask. This aliquot part is equivalent to $^3/_4$ of the original sample or, in this instance, about three grains of phenobarbital. Titrate the clear solution with 0.1*N* silver nitrate solution, using a 10-cc. burette, until the first distinct turbidity is evident and one that persists after thoroughly shaking. The silver nitrate solution must be added very slowly and toward the end-point must be added drop by drop shaking vigorously after the addition of each drop. The immediate turbidity caused by the addition of each drop must be cleared by shaking before the addition of the next drop, until the final end-point is reached which is characterized by the persistent turbidity of the solution even after vigorous shaking. The titrated solution at this stage begins to change rapidly to a brownish black color due to the separation of the silver oxide. Each cc. of 0.1*N* silver nitrate solution is equivalent to 0.023212 Gm. of phenobarbital.

The results of a typical series of determinations of phenobarbital and theobromine in Theocalcin mixtures are shown in Table II. It will be noted that the determinations were made in triplicate by two different operators. The known samples were carefully made to contain the same ratio of Theocalcin and phenobarbital as the commercial product; namely, $7^1/_2$ grains of Theocalcin to $^1/_4$ grain of phenobarbital.

Table II.—Results of Analyses of Theocalcin-Phenobarbital Mixtures

		Theobromine in Theocalcin			Phenobarbital		
Operator	Sample	Number of Grains in Sample	Number of Grains Found	Per Cent of Recovery	Number of Grains in Sample	Number of Grains Found	Per Cent of Recovery
A	1	7.19	7.18	99.86	3.000	2.988	99.60
	2	7.19	7.18	99.86	3.000	3.026	100.87
	3	7.19	7.17	99.72	3.000	2.981	99.37
B	1	7.20	7.19	99.86	3.000	2.967	98.90
	2	7.20	7.19	99.86	3.000	2.939	97.97
	3	7.20	7.19	99.86	3.000	2.991	99.68

II. Mixtures of Theobromine and Phenobarbital.—Although Method I is recommended for most mixtures of theobromine and phenobarbital a somewhat simplified method may be employed for separating the components of simple mixtures—namely, by the use of a continuous extraction apparatus with alcohol-free ether as a solvent. The modified methods follow below.

Theobromine.—Accurately weigh a sample of the mixture corresponding to about ten grains of theobromine and transfer it to a small paper extraction thimble. Connect to a simple continuous extraction apparatus of the rubber extraction type using about 75 cc. of alcohol-free ether as a solvent. Extract for about thirty minutes and discard the ether containing the phenobarbital. Place the paper extraction thimble containing the theobromine in a 500-cc. wide-mouth Erlenmeyer flask which contains 150 cc. of water and 25 cc. of approximately 0.1*N* sulfuric acid solution. Allow the paper thimble to disintegrate in this solution or carefully tear it apart using a stirring rod. Boil the solution vigorously for one to two minutes and then cool to about 40° C. Add 1½ cc. of Phenol-red solution (0.02%) and make the solution distinctly alkaline with a slight excess (1–2 cc.) of 0.1*N* sodium hydroxide solution. Then adjust the solution by the careful addition, drop by drop, of 0.1*N* sulfuric acid solution until the blusih red color is discharged and the first distinctly yellow color is produced. Then add about 50 cc. of approximately 0.1*N* silver nitrate solution and titrate the liberated nitric acid slowly with 0.1*N* sodium hydroxide until the solution just acquires a bluish red color. Each cc. of 0.1*N* sodium hydroxide solution consumed in the neutralization of the nitric acid (that is, after the addition of the solution of silver nitrate) is equivalent to 0.01801 Gm. of theobromine.

Phenobarbital.—Accurately weigh a sample of the mixture corresponding to about four grains of phenobarbital, and transfer it to a paper extraction thimble. Connect to a simple extraction apparatus as described above using 100 cc. of alcohol-free ether. Extract for thirty minutes and discard the paper thimble containing the theobromine. The ether, which contains the phenobarbital, is distilled off on a water bath until 2 to 3 cc. remain. The last trace of ether is allowed to evaporate at room temperature or not over 40° C. The residue is dissolved in exactly 100 cc. of a solvent composed of 45 cc. of ethyl alcohol, 30 cc. of water and 25 cc. of 1.0*N* sodium hydroxide solution. (The components of this solvent must be mixed and cooled to room temperature before adding to the phenobarbital extract.) After the phenobarbital is dissolved, the solution is filtered through a quantitative grade of filter paper, discarding the first ten cc. of the filtrate. Transfer exactly 75 cc. of the filtrate, which must be perfectly clear, to a 250-cc. Erlenmeyer flask; this aliquot part is equivalent to ¾ of the original sample or, in this instance, about three grains of phenobarbital. Titrate the clear solution with 0.1*N* silver nitrate solution until the first distinct and persistent turbidity results, using the precautions as outlined for the determination of phenobarbital in mixtures with Theocalcin which is outlined above. Each cc. of 0.1*N* silver nitrate solution is equivalent to 0.023212 Gm. of phenobarbital.

The results of a typical series of determinations of theobromine and phenobarbital in mixtures are shown in Table III. The determinations were made in triplicate by two different operators on samples containing two different ratios of theobromine and phenobarbital; one series of samples contained a ratio of 5 grains of theobromine to ¼ grain of phenobarbital whereas the other series contained a ratio of 5 grains of theobromine to ½ grain of phenobarbital.

Instead of using a solvent composed of ethyl alcohol, sodium hydroxide solution and water for dissolving the extracted phenobarbital, it is possible to substitute acetone for the ethyl alcohol. In this event the solvent should be composed of 25 cc. of 1.0*N* sodium hydroxide solution, 35 cc. of acetone and 40 cc. of distilled water per hundred cc. of solvent in the case of theobromine-phenobarbital mixtures. An attempt was made to use a dilute (3⅓%) solution of sodium carbonate for dissolving the extracted phenobarbital but the results were inconsistent for some unexplained reason, perhaps because of the indefinite end-point.

Because of the small amount of phenobarbital in the sample and the relatively large phenobarbital equivalent when using 0.1*N* silver nitrate solution,

Table III.—Results of Analyses of Theobromine-Phenobarbital Mixtures

		Theobromine			Phenobarbital		
Operator	Sample	Number of Grains in Sample	Number of Grains Found	Per Cent of Recovery	Number of Grains in Sample	Number of Grains Found	Per Cent of Recovery
A	1	10.00	9.970	99.70	3.000	2.964	98.80
	2	10.00	9.984	99.84	3.000	2.964	98.80
	3	10.00	9.960	99.60	3.000	3.019	100.63
	4	10.00	9.900	99.00	6.000	5.962	99.37
	5	10.00	10.000	100.00	6.000	6.018	100.30
	6	10.00	10.060	100.60	6.000	5.982	99.70
B	1	10.00	9.986	99.86	3.000	3.008	100.27
	2	10.00	10.000	100.00	3.000	3.002	100.07
	3	10.00	9.986	99.86	3.000	2.973	99.10
	4	10.00	10.018	100.18	6.000	5.926	98.77
	5	10.00	10.018	100.18	6.000	5.965	99.42
	6	10.00	10.028	100.28	6.000	5.990	99.83

an attempt was made to use 0.05*N* silver nitrate solution. Again the end-point was rather indefinite and therefore the results were inconsistent.

The common tablet diluents, such as starch and talcum, were used in making up the experimental samples and they offered no special difficulties. However, stearic acid and its salts were found to interfere with the phenobarbital determination due to reaction with silver nitrate.

The outlined methods for the determination of theobromine in mixtures with phenobarbital are very accurate as shown by the results recorded in Tables II and III but there are certain precautions which should be observed: the titration must be carried out at a slow rate, about two to three cc. per minute during the beginning of the titration and drop by drop toward the end-point; carbon dioxide must be removed from the solution by boiling; the volumetric solutions should be standardized using phenol red as an indicator; and finally the operator should acquire experience in detecting the end-point by running several samples of pure theobromine alkaloid.

Although the method outlined for the determination of phenobarbital in mixtures is slightly long and requires some skill, it gives reasonably good results as shown by Tables II and III. Again there are certain precautions which must be observed: it is important that no alcohol be present in the ether employed for the extraction of the phenobarbital; the alkalinity of the solution of extracted phenobarbital at the time of titration should not be varied appreciably from the figures mentioned because the sharpness of the end-point is influenced by this factor; since only a small amount of silver nitrate solution is used in the titration a small burette (usually 10 cc. size) graduated in 0.05 cc. should be used; and most important of all, the experience and technique of the operator are very important. It is absolutely essential that the operator familiarize himself with the method, particularly the end-point, by running several samples of pure phenobarbital. It should be pointed out again that the success of the method depends largely on the skill and experience of the operator.

In conclusion, it might be stated that the outlined methods have been used successfully by this laboratory for a period of two years and have also been successfully applied to mixtures of other manufacturers.

SUMMARY

1. The literature does not show a method for the determination of theobromine, or theobromine salts, and phenobarbital in mixtures, but the literature is briefly reviewed for methods of determining theobromine and phenobarbital individually.

2. A method is described for the determination of theobromine in mixtures with phenobarbital which consists essentially of separating or removing the phenobarbital, forming silver theobromine and liberating nitric acid quantitatively by the use of a silver nitrate solution. The nitric acid is then titrated with standard alkali.

3. A method is described for the determination of phenobarbital in mixtures with theobromine or theobromine salts which consists essentially of first separating the phenobrabital and using a silver nitrate solution to form insoluble silver phenobarbital which in a dilute sodium hydroxide solution is extremely weakly disassociated so that the remainder of the sodium hydroxide solution causes a separation of silver oxide gradually and incompletely.

4. The solubilities of both theobromine and phenobarbital in various solvents are tabulated.

5. The results of a series of determinations are tabulated to show the dependability of the methods. Samples were used which contained theobromine or Theocalcin and phenobarbital in the ratios normally found. Eighteen determinations of phenobarbital in various samples averaged 99.75% recovery and eighteen determinations of theobromine in various samples averaged 99.90% recovery.

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REFERENCES

(1) The National Formulary, 6th Edition, Mack Printing Company, Easton, Pa. (1936), page 381.

(2) Montignie, E., *Bull. soc. chim.* (5), 2 (1935), 373–375.

(3) Bougault and Guillou, *Compt. rend.*, 193 (1931), 463–466.

(4) Fleury, Paul, *Bull. soc. chim.*, 37 (1925), 1656–1668.

(5) Zwikker, *Pharm. Weekblad.*, 68 (1931), 975.

(6) Dille & Koppanyi, *Jour. A. Ph. A.*, 23 (1934), 1079.

(7) Babick, S., *Pharm. Monatsch.*, 17 (1936), 87.

(8) Budde, Hans, *Apoth. Ztg.*, 49 (1934), 295.

(9) Kalinowski, Kazimierzi, *Wiadomości Farm.*, 62 (1935), 633–635 & 649–657.

(10) Pharmacopœia of the United States, XI Revision, Mack Printing Co., Easton, Pa. (1936), page 383.

(11) Emery, W. O., and Spencer, G. C., *Ind. Eng. Chem.*, 10, No. 8 (1918), 605–606.
(12) Matthes and Schutz, *Pharm. Ztg.*, 75 (1930), 42–44.
(13) Saiko, Berta-Pittner, *Pharm. Monatsch.*, 11 (1930), 73.
(14) Miko, Gyula, *Arch. pharm.*, 270 (1932), 163–167.
(15) Van Breukelevean, M., *Chem. Weekblad*, 24 (1927), 206–207.
(16) Boie, Heinrich, and Linder, Alois, *Pharm. Ztg.*, 74 (1929), 418–420.
(17) Boie, Heinrich, *Ibid.*, 75 (1930), 968–969.
(18) Jorgensen, P. S., *Jour. A. O. A. C.*, 21 (1938), 555–557.
(19) Van Giffen, H. J., *Pharm. Weekblad*, 69 (1932), 1321–1325.
(20) Methods of Analysis of A. O. A. C., 5th Edition (1940), page 593.

Note on the Behavior of Sulfonamides in the Cobalt Color Tests for Barbiturates

*By Theodore Koppanyi, Melvin W. Green and Charles R. Linegar**

Recently, samples of sulfathiazole contaminated with phenobarbital were found on the open market. In connection with this finding, Dr. Knudson of the Albany Medical College and others called our attention to the fact that the Koppanyi test for barbiturates is also positive to the sulfonamides. In the fear that some unadulterated sulfonamide preparation might be rejected, unfairly, on the basis of this color test, we have investigated the behavior of sulfathiazole, sulfani'amide and sulfapyridine with reference to the cobalt color tests. We are concerned here with two different aspects of the same problem: (1) the differentiation of barbiturates from sulfonamides in pharmaceutical analytical work, and (2) the possible interference of sulfonamides, in the urine and other body fluids, in the diagnosis of barbiturate poisoning.

EXPERIMENTAL

1. Dille and Koppanyi (1) have shown that the cobalt acetate-isopropylamine test may be used to assay pharmaceutical preparations containing barbiturates. In that procedure the alkaline preparation is dissolved in water, acidulated and then shaken out with at least ten volumes of chloroform.

The same procedure was followed using prepared mixtures containing known amounts of phenobarbital and different sulfonamides.

Table I indicates that the presence of sulfonamides does not appreciably interfere with the pharmaceutical assay of barbiturate preparations provided the Dille-Koppanyi procedure is strictly adhered to. The sample containing 100 mg. of sulfonamides with no barbiturates yields less color, in the colorimetric procedure, than does 2.0 mg. of phenobarbital. The presence of phenobarbital or any barbiturate in admixture with any suspected sulfonamide preparation can thus be detected by the following procedure. Weigh out a 100-mg. sample of the suspected sulfonamide, dissolve in 20 cc. of water, acidulate and shake out with 20 volumes of chloroform. Simultaneously, subject a 100-mg. sample of the pure sulfonamide to the same procedure. Evaporate the chloroform extract to dryness and take up the residue in a convenient, measured volume of chloroform. Compare each of these chloroform solutions with suitable standard solutions (0.02 to 0.08 per cent in chloroform) of the barbiturate in question. If the suspected sulfonamide produces a more intense color than the pure sulfonamide itself, the preparation is contaminated. And, moreover, the degree of adulteration can be

Table I.—Recovery of Phenobarbital from Phenobarbital-Sulfonamide Mixtures as Determined by the Cobalt-Isopropylamine Method

Sulfonamides Used	Amount of Sulfonamide in Sample, Mg.	Amount of Phenobarbital in Sample, Mg.	Color Comparison in Terms of Pure Phenobarbital as a Standard, Mg.	Percentage Phenobarbital Recovery, Per Cent
Sulfathiazole	100	0.0	<2.0–	.....
Sulfathiazole	95	5.0	4.6– 5.7	91.2–114
Sulfathiazole	90	10.0	9.2–10.4	92.4–104
Sulfathiazole	50	50.0	54.0–56.7	108.0–113.4
Sulfapyridine	100	0.0	<2.0–	.....
Sulfapyridine	50	50.0	54.5	109.0
Sulfapyridine[a]	50	50.0	58.0	116.0

[a] Determination made in the chloroform solution directly.

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