# Application of Magnesium Silicate Adsorption Chromatography to the Determination of Mephobarbital and Diphenylhydantoin in Tablets

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**Abstract** □ A quantitative chromatographic separation followed by UV spectrophotometric determination is presented for the analysis of a commercial tablet formulation containing mephobarbital and diphenylhydantoin. A patented activated magnesium silicate is used for the chromatographic column separation. Mephobarbital is eluted with 4% ethyl acetate in chloroform and diphenylhydantoin is eluted with 10% methanol in chloroform.

**Keyphrases** □ Mephobarbital, diphenylhydantoin tablets—analysis □ Column chromatography—separation □ UV spectrophotometry—analysis

Two anticonvulsant drugs, mephobarbital and diphenylhydantoin, are contained in a commercial tablet formulation prescribed for the treatment of epilepsy. In the standard methods for analysis of each individual drug, published in the NF (1, 2), mephobarbital in tablets is determined gravimetrically and diphenylhydantoin volumetrically. Since these two drugs have similar chemical properties, *i.e.*, they both are *N,N'*-distributed cyclic ureide acids, the individual assay procedures are not suitable for analysis of the mixture.

Dille and Koppanyi (3) have published colorimetric methods for the spectrophotometric determination of barbiturates as colored cobalt salts. Hydantoins also form colored cobalt salts under the same conditions (4). Similar preparations containing phenobarbital and diphenylhydantoin have been separated and determined by Marino (5), but mephobarbital and diphenylhydantoin were not quantitatively separated when Marino's method was applied.[1]

Westerink (6) separated mephobarbital and diphenylhydantoin on a buffered diatomaceous earth column[2] with subsequent determination by UV spectrophotometry. He encountered errors as high as 5% for each drug. Attempts to determine diphenylhydantoin in basic media were unsuccessful, since diphenylhydantoin does not exhibit a quantitative maximum in the UV region in basic solutions (7).

Stainer and Daube (8) determined mephobarbital in the presence of diphenylhydantoin without separation. Mephobarbital and diphenylhydantoin have been determined spectrophotometrically in the presence of each other by solving simultaneous equations and by compensatory procedures.[1] In the proposed procedure, mephobarbital and diphenylhydantoin are separated on a chromatographic column containing an activated mixture of magnesium oxide, silicon dioxide, and sodium sulfate; this adsorbent also is used in the analysis of pesticides, vitamins, alkaloids, steroids, and other compounds (9). The separated compounds are determined by UV spectrophotometry. Mephobarbital is eluted from the column with 4% ethyl acetate in chloroform and diphenylhydantoin with 10% methanol in chloroform. Mephobarbital is determined in dilute sodium hydroxide solution and diphenylhydantoin in dilute sulfuric acid-methanol solution.

A commercially available tablet,[3] with a label declaration of 90 mg. of mephobarbital and 60 mg. of diphenylhydantoin, was the test preparation used in this study.

## EXPERIMENTAL

**Apparatus**—A recording UV spectrophotometer with quartz 1.0-cm. matched cells.

**Reagents**[4]—*Solvents*—Chloroform (containing 1% ethanol as a preservative), methanol, and ethyl acetate.

*Adsorbent*—Patented activated magnesium silicate.[5]

*Acid Methanol Solution*—Dilute 20 ml. of 10% (w/v) sulfuric acid in water to 200 ml. with methanol.

*Sodium Hydroxide Solution*—Prepare 0.01 *N* solution by dissolving 0.2 g. of sodium hydroxide in 500 ml. of water.

*Eluting Solutions*—Solution A: 4% (v/v) ethyl acetate in chloroform; solution B: 10% (v/v) methanol in chloroform.

*Standard Solutions*—Dissolve 125 mg. of mephobarbital in chloroform and dilute to 100 ml.; dilute a 10.0-ml. aliquot to 100 ml. with chloroform. Dissolve 83 mg. of diphenylhydantoin in acid-methanol solution and dilute to 100 ml.

**Sample Preparation**—Grind a representative number of tablets to pass a 60-mesh sieve. Accurately weigh a portion of the powder equivalent to 125 mg. of mephobarbital, and transfer to a standard taper conical flask. Add 90 ml. of chloroform and reflux with magnetic stirring for 1 hr. with intermittent swirling.[6] Cool the solution to room temperature and pour into a 100-ml. volumetric flask. Rinse the conical flask with small amounts of chloroform. Dilute to volume with chloroform.

**Chromatographic Column Preparation**—Transfer 5.0 g. of the adsorbent to a chromatographic column 250 mm. long and 10.5 mm. i.d., having a fritted disk and a polytetrafluoroethylene stopcock.[7] Tap the column wall gently to settle the adsorbent. Insert a small cotton plug above the adsorbent.

**Chromatographic Separation**—Place a 100-ml. volumetric flask under the column. Pipet a 10.0-ml. aliquot of the sample preparation onto the column. Pass the sample solution into the cotton and wash the inner column wall with a small amount of chloroform to insure complete placement of the sample on the adsorbent. After the wash has penetrated the cotton, elute to approximately 98 ml. with eluting Solution A at a rate of 2 drops/sec. Close the column stopcock, leaving a small amount of liquid above the cotton.

[1] Analysis performed by analysts in the FDA Buffalo District laboratory.

[2] Celite, Johns Manville, New York, N. Y.

[3] Mebroin is a registered trademark of Winthrop Laboratories, New York, N. Y.

[4] All inorganic reagents used were reagent grade; organic solvents were spectral grade.

[5] Florisil, 60-100 mesh, Fisher Chemical Co., Cat. No. F-100.

[6] The sample may also be prepared by placing the weighed sample directly into a 100-ml. volumetric flask, adding 90 ml. of chloroform, and vibrating the volumetric flask in the bath of an ultrasonic generator for 5 min. with intermittent shaking. Fine particles and colored insoluble material do not interfere.

[7] Teflon, E. I. du Pont de Nemours & Co., Wilmington, Del.

Table I—Analyses of the Synthetic Mixture

| Analysis No. | Mephobarbital Recovered, mg. | Diphenylhydantoin Recovered, mg. |
|---|---|---|
| 1 | 122.0 | 86.0 |
| 2 | 121.6 | 85.6 |
| 3 | 120.9 | 86.7 |
| 4 | 122.6 | 86.4 |
| 5 | 122.8 | 85.8 |
| 6 | 122.6 | 85.1 |
| 7 | 122.6 | 85.8 |
| 8 | 121.9 | 85.9 |
| 9 | 121.7 | 86.5 |
| *SD*, mg. recovered | 122.1 ± 0.6 | 86.0 ± 0.5 |
| *SD*, % recovered | 98.4 ± 0.5 | 101.7 ± 0.6 |

Wash the column tip and dilute to volume with chloroform. Reserve this eluate for the determination of mephobarbital.

Place a 50-ml. volumetric flask under the column. Elute with eluting Solution B at a rate of 2 drops/sec., collecting approximately 48 ml. Wash the column tip and dilute to volume with methanol. Reserve this solution for the determination of diphenylhydantoin.

**Spectrophotometry**—*Mephobarbital*—Evaporate a 10.0-ml. aliquot of the Solution A eluate and a 10.0-ml. aliquot of the diluted standard to approximately 1 ml. of solution with a current of air on a steam bath and to dryness with air alone. Dissolve the residues in 50.0 ml. of 0.01 *N* NaOH. Record the UV spectra of the sample and the standard from 300 to 220 mμ. Subtract any background absorbance at 300 mμ from the maximum absorbance at 245 mμ.

*Diphenylhydantoin*—Evaporate a 25.0-ml. aliquot of the Solution B eluate to approximately 1 ml. on steam bath with a current of air and to final dryness with air alone. Dissolve the residue in 10.0 ml. of acid-methanol solution. Record the UV spectra of the standard and sample from 280 to 260 mμ in a 1.0-cm. cell against acid-methanol solution. Subtract any background absorbance at 280 mμ from the maximum absorbance at 265 mμ.

Calculate the concentration of mephobarbital and diphenylhydantoin in the sample by comparing the absorbance of the standard and sample at the particular wavelengths indicated.

## RESULTS AND DISCUSSION

The accuracy of the proposed method is based upon nine replicate analyses of a synthetic composite solution containing 124 mg. of mephobarbital and 84.6 mg. of diphenylhydantoin. Recoveries for each drug and the statistical evaluation of the results are presented in Table I.

Five different lots of the commercial preparation were assayed in quadruplicate by the proposed method. The results of these determinations and the statistical treatment of the data are presented in Table II.

The replicate results from the synthetic mixture and commercial product analyses were used to statistically determine the precision of the method.

Mephobarbital and diphenylhydantoin were added to three previously assayed samples of the commercial product, and the mixtures were reassayed by the proposed method. Recoveries of the added drugs are listed in Table III.

The absorptivity of diphenylhydantoin was affected by the pH of the medium in which the absorbance was determined. Spectra of the sample and standard showed distinct quantitative maxima in acid-methanol solution. The quantitative aspects of the UV spectra were destroyed when the acidic solution was made basic.

Absorptivities were calculated on all standard curves of mephobarbital and diphenylhydantoin. Values obtained were 355 ± 2 for mephobarbital and 166 ± 0.2 for diphenylhydantoin. Mephobarbital was found to be stable in chloroform for at least 1 day and in 0.01 *N* sodium hydroxide for at least 30 min., and diphenylhydantoin in acid-methanol solution for at least one day.

No difficulties were encountered when fresh lots of the adsorbent were used. If the adsorbent was exposed to the air or to moisture for any length of time, the adsorbent became deactivated. This resulted in high mephobarbital and low diphenylhydantoin recovery values. When the wet or deactivated adsorbent was dried at 100° for a short period of time, the original activity was restored. When the adsorbent was dried at temperatures greater than 100°, it became too active. This resulted in low mephobarbital and high diphenylhydantoin recovery values.

Separation of phenobarbital and diphenylhydantoin, a more common mixture, was not investigated by the proposed procedure.

Table II—Analysis of the Commercial Product

| Code | Analysis No. | Mephobarbital, mg./tab. found | Diphenylhydantoin, mg./tab. found |
|---|---|---|---|
| A | 1 | 91.6 | 60.2 |
| A | 2 | 88.4 | 59.4 |
| A | 3 | 90.6 | 60.5 |
| A | 4 | 88.9 | 59.8 |
| | *SD*, mg./tab. | 88.9 ± 1.5 | 59.7 ± 0.4 |
| | *SD*, % of declaration | 98.8 ± 1.6 | 99.5 ± 0.7 |
| B | 5 | 88.4 | 58.7 |
| B | 6 | 88.6 | 57.4 |
| B | 7 | 88.4 | 58.4 |
| B | 8 | 88.1 | 59.1 |
| | *SD*, mg./tab. | 88.4 ± 0.2 | 58.4 ± 0.6 |
| | *SD*, % of declaration | 98.2 ± 0.2 | 97.3 ± 1.0 |
| C | 9 | 89.2 | 61.1 |
| C | 10 | 88.0 | 60.8 |
| C | 11 | 88.4 | 61.2 |
| C | 12 | 87.6 | 61.1 |
| | *SD*, mg./tab. | 88.3 ± 0.7 | 61.0 ± 0.2 |
| | *SD*, % of declaration | 98.1 ± 0.8 | 101.7 ± 0.3 |
| D | 13 | 90.2 | 60.0 |
| D | 14 | 90.0 | 59.8 |
| D | 15 | 88.4 | 59.5 |
| D | 16 | 89.6 | 59.7 |
| | *SD*, mg./tab. | 89.6 ± 0.8 | 59.7 ± 0.2 |
| | *SD*, % of declaration | 99.6 ± 0.9 | 99.7 ± 0.3 |
| E | 17 | 87.6 | 59.5 |
| E | 18 | 88.0 | 58.9 |
| E | 19 | 89.1 | 57.0 |
| E | 20 | 89.5 | 59.2 |
| | *SD*, mg./tab. | 88.6 ± 0.9 | 58.7 ± 1.1 |
| | *SD*, % of declaration | 98.4 ± 1.0 | 97.8 ± 1.8 |
| | Av. *SD*, mg./tab. | 88.8 ± 0.8 | 59.5 ± 0.5 |
| | Av. *SD*, % of declaration | 98.6 ± 0.7 | 99.2 ± 0.8 |

Table III—Recoveries of Drugs Added to the Commercial Product

| Sample | Mephobarbital | | | Diphenylhydantoin | | |
|---|---|---|---|---|---|---|
| | mg. Added | mg. Recovered | % Recovery | mg. Added | mg. Recovered | % Recovery |
| A | 58.7 | 58.2 | 99.1 | 42.7 | 43.3 | 101.4 |
| B | 47.2 | 46.5 | 98.5 | 45.4 | 45.1 | 99.3 |
| C | 32.0 | 31.8 | 99.4 | 27.8 | 27.5 | 98.9 |
| *SD*, % recovery | | | 99.0 ± 0.5 | | | 99.9 ± 1.3 |

One sample of phenobarbital was eluted quantitatively from the adsorbent with diethyl ether containing 2% ethanol as a preservative. Prior experimental data showed that diphenylhydantoin was not eluted from the adsorbent with diethyl ether. Preparations containing sodium salts of phenobarbital and/or diphenylhydantoin would probably have to undergo acidification and extraction before column separation.

Mephobarbital and diphenylhydantoin recoveries were determined individually before synthetic mixtures were prepared. Recoveries were 98–99% for 80–115 mg. of mephobarbital and 99–101% for 40–100 mg. of diphenylhydantoin. Synthetic mixtures containing 90–150 mg. of mephobarbital and 40–100 mg. of diphenylhydantoin were then separated. Recoveries were 98 ± 0.5% for mephobarbital and 102 ± 1% for diphenylhydantoin. A small amount of mephobarbital tailing enhanced the background absorbance of diphenylhydantoin, resulting in slightly high diphenylhydantoin recoveries and low mephobarbital recoveries. This tailing could not be reduced experimentally, but its effect was minimized by subtracting the background absorbance. When recoveries were calculated with the corrected absorbances, recovery values for mephobarbital and diphenylhydantoin approached closest to 100%.

## SUMMARY

An activated magnesium silicate has been used successfully to separate mephobarbital and diphenylhydantoin in a commercial tablet formulation. Application of this adsorbent to the determination of other mixtures may also yield favorable separations.

## REFERENCES

(1) "The National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965, p. 235.

(2) *Ibid.*, p. 141.

(3) F. D. Snell and C. T. Snell, "Colorimetric Methods of Analysis," Vol. IV, 3rd ed., D. Van Nostrand, Princeton, N. J., 1959, pp. 92–103.

(4) "The National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965, p. 142.

(5) V. S. Marino, *J. Assoc. Offic. Agr. Chemists*, **48**, 582(1965).

(6) D. Westerink, *Pharm. Weekblad*, **97**, 849(1962).

(7) I. Sunshine and S. R. Gerber, "Spectrophotometric Analysis of Drugs," Charles C. Thomas, Springfield, Ill., 1963, p. 49.

(8) C. Stainer and Y. Daube, *Duychserte-Bull. Acad. Roy Med. Belg.*, **1**, 583(1961); through *Chem. Abstr.*, **56**, 7433d(1962).

(9) "*Florisil*," Floridin Co., advertisement pamphlet, Pittsburgh, Pa.

## ACKNOWLEDGMENTS AND ADDRESSES

Received April 24, 1968, from the *U. S. Department of Health, Education, and Welfare, Food and Drug Administration, Buffalo District, Buffalo, NY 14202*

Accepted for publication March 11, 1969.

# Determination of Niacinamide in Pharmaceutical Preparations

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**Abstract** □ A method for the isolation of niacinamide from multicomponent pharmaceutical preparations by partition chromatography between a diatomaceous earth-water column and chloroform followed by measurement of UV absorbance at 262 mμ is proposed. A second method using the color produced by the niacinamide in the column eluate with bromothymol blue in chloroform is also proposed to allow measurements in the visible range. The chromatographic separation is specific for niacinamide and the procedures are simple and rapid. However, alcohol modifies the partition between water and chloroform, causing the niacinamide to be eluted early. The *SD* of the UV absorbance method is 0.5% and of the bromothymol blue method is 1.7%. The precision of the proposed UV absorbance method compares favorably with that of the Pelletier and Campbell method and gives values approximately 2% higher.

**Keyphrases** □ Niacinamide in multicomponent products—analysis □ Partition chromatography—separation □ UV spectrophotometry—analysis □ Colorimetric analysis—spectrophotometer □ Bromothymol blue—color reagent □ Cyanogen Br-barbituric acid—color reagent

Niacinamide is an important ingredient in a variety of multicomponent pharmaceutical preparations and the great diversity of these mixtures requires a specific analytical procedure to insure accurate results. Most of the photometric methods of determination now in use are based on the Koenig reaction (1, 2) of pyridine and its derivatives with cyanogen bromide and an aromatic amine. The AOAC (10th ed.) method (3) uses sulfanilic acid and is primarily utilized for the determination of total nicotinic acid in natural products. The Pelletier and Campbell method (4) as modified by Pelletier (5) utilizes barbituric acid. Up to three times as much niacin does not interfere with this method, which has been adopted as official, first action, by the AOAC (6). The UV spectrum may also be used but is restricted to solutions containing only niacinamide unless interferences can be eliminated (7) by ion-exchange or other procedures. Polarographic (8) and GLC (9) methods have also been proposed as specific methods. All these methods suffer from disadvantages such as the use of noxious cyanogen bromide, low color stability, poor reproducibility, and interferences by other components of multivitamin preparations.

This paper reports two methods using column chromatographic separation followed by measurement of the UV absorbance of niacinamide in 0.1 *N* HCl or of the color developed by niacinamide with bromothymol blue in chloroform solution. The methods are simple, specific, and show accuracy and precision equivalent to or better than the cyanogen bromide methods.