Determination of Phenaglycodol in Biologic Specimens by Ultraviolet Spectrophotometry

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A rapid spectrophotometric method for the determination of phenaglycodol in body fluids and tissues is presented. Separation from other drugs is not required. The substituted glycol is oxidized by means of acid dichromate to a carbonyl derivative which has a high molar absorptivity in the ultraviolet region. The carbonyl product is converted to a semicarbazone which exhibits an even greater sensitivity to the absorption of ultraviolet radiations. The spectra of the phenaglycodol derivatives permit enough specificity for use of the procedure in forensic chemistry. The method is sufficiently sensitive for determination of phenaglycodol in urine 5 days after the ingestion of a single therapeutic dose. The distribution pattern of phenaglycodol in the rat and the nature of its excretion in man are presented.

PHENAGLYCODOL[1] (2-*p*-chlorophenyl-3-methyl-2, 3-butanediol) is claimed to have a distinct tranquilizing effect. Its calming and muscle relaxing properties are probably comparable to that of meprobamate (1, 2). The literature contains few methods for the analysis of this drug. Rose (3) described a technique for determining phenaglycodol by electrolytic cleavage of the glycol group to yield *p*-chloroacetophenone, which has a sensitive ultraviolet absorption spectrum. The procedure, however, is not applicable to determination of the drug in biologic specimens. Cima and Fassina (4) have published a method that utilizes both chromatographic and colorimetric techniques for determining phenaglycodol in urine. However, the method is applicable only to urine and the colorimetric assay, which utilizes a phosphomolybdic reagent, is subject to interference by many carbohydrates and polyphenols. Goldbaum (5) determined neutral drugs by gas chromatography, but his procedure does not provide positive identification nor does it afford routine application in the analysis of biologic specimens. In addition, no mention of phenaglycodol is made. In hexane, phenaglycodol absorbs radiation strongly at 222 mμ; therefore, small amounts of the drug in pure solution can be determined by spectrophotometric analysis (6). In biologic extracts, however, the ultraviolet spectral curve of the drug at 222 mμ is so affected by background absorption that direct analysis of unconverted drug, even in high concentrations, is not feasible. In addition, the solubility characteristics of this drug make difficult any procedure for obtaining it in an environment suitable for direct spectrophotometric assay. The need for a specific quantitative method for determining phenaglycodol in body fluids and tissues is apparent.

This report describes a rapid and specific method for the quantitative determination of phenaglycodol. Separation of phenaglycodol from other neutral drugs is not required. The method permits a reliable evaluation of the drug intake by patients receiving treatment with phenaglycodol.

EXPERIMENTAL

Instrumentation—A Beckman DK-2A ratio-recording spectrophotometer with linear presentation was used for the ultraviolet absorption measurements. A sample path length of 10 mm. was used throughout the study. Infrared functional group assignments were made with a Beckman IR 4 spectrophotometer.

Method—At any pH phenaglycodol is extracted from blood, urine, and tissues into most organic solvents. In this procedure 10 ml. of whole oxalated blood or serum or 10 Gm. of homogenized tissue is shaken vigorously for 3 to 5 min. with 100 ml. of chloroform. For urine, 10 ml. of specimen is refluxed for 10 min. with 1 ml. of concentrated hydrochloric acid prior to chloroform extraction. After the two phases have separated, the chloroform layer is removed and filtered through Whatman No. 41 or equivalent filter paper. The filtrate is subsequently washed with 10 ml. of 1 *N* sodium hydroxide solution and then with 10 ml. of 1 *N* hydrochloric acid solution. The volume of washed chloroform is recorded and the amount lost is considered in the final calculations. The chloroform is subsequently evaporated to dryness at 50° under vacuum. To the residue are added 20 ml. of 0.1 *N* potassium dichromate in 10 *N* sulfuric acid and 50 ml. of spectroquality hexane. The contents are refluxed for 30 min. with constant mechanical stirring, after which the hexane layer is cooled, separated, and read in a spectrophotometer against a hexane blank. When moderate or low levels of the drug are indicated, conversion of the reaction product to a semicarbazone derivative is desirable. This is accomplished by reacting 4–40 ml. of the hexane containing reaction product with 4 ml. of 0.5 *M* semicarbazide hydrochloride buffered to pH 3.5 with sodium acetate. The reaction is done conveniently at room temperature in a suitable size flask connected to a vacuum

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[1] Marketed as Ultran by Eli Lilly and Co., Indianapolis, Ind.

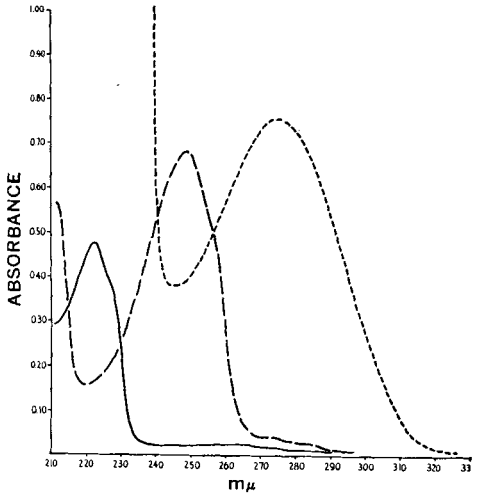

Fig. 1—Ultraviolet absorption spectra of phenaglycodol in hexane, of the phenaglycodol reaction product in hexane, and of the semicarbazone of the reaction product in water, each corresponding to a phenaglycodol concentration of 8 mcg./ml. Key: ——, phenaglycodol; — —, phenaglycodol product; ---, semicarbazone of phenaglycodol product.

TABLE I—STANDARD CURVE OF PHENAGLYCODOL REACTION PRODUCT

Phenaglycodol in Sample, mcg./ml.	Absorbance of Reaction Product[a]	Absorbance/ Concn.	Absorbance of Semicarbazone of Reaction Product[b]	Absorbance/ Concn.
10	0.843	0.084	0.954	0.095
8	0.700	0.087	0.739	0.093
6	0.500	0.083	0.589	0.098
4	0.323	0.081	0.366	0.092
2	0.168	0.084	0.201	0.101
Av.		0.084		0.096

[a] In hexane. [b] In aqueous semicarbazide.

rotary evaporator. Semicarbazone formation is complete when all the hexane has been removed. The remaining aqueous layer is read at 273 mμ against a 0.5 *M* semicarbazide blank.

The amount of phenaglycodol in the original sample is calculated from a standard curve prepared from aqueous solutions of the drug in appropriate concentrations, which have been carried through the procedure described, including the concentration technique for solutions containing small amounts of the drug.

RESULTS AND DISCUSSION

The ultraviolet absorption curve of the reaction product of phenaglycodol is well defined. It has an absorption maximum at 247 mμ in hexane and at 254 mμ in water. The unconverted drug absorbs maximally at 222 mμ. The high absorbance at 247 mμ for the reaction product and at 273 mμ for the semicarbazone of the product (Fig. 1), provides increased sensitivity and specificity over that of techniques which rely on direct spectrophotometric analysis of unconverted phenaglycodol. At 247 mμ a linear relationship exists between the absorbance of the reaction product and the concentration of the drug in the original sample. At 273 mμ a similar relationship exists for the semicarbazone of the product (Table I). Phenaglycodol extracted from urine of man and from tissues of animals that have received the drug yields reaction products that have identical ultraviolet absorption spectra to that obtained from the pure drug.

Infrared spectra of phenaglycodol (Fig. 2) and its reaction product (Fig. 3) show several distinct differences, of which the most significant is a band at 1692 cm.$^{-1}$ in the spectrum of the product. Absorption at this wavelength is entirely absent in the infrared spectrum of phenaglycodol. This band is believed to be the result of a carbonyl group formed by dichromate oxidation of the glycol group in the parent compound. The conversion of the

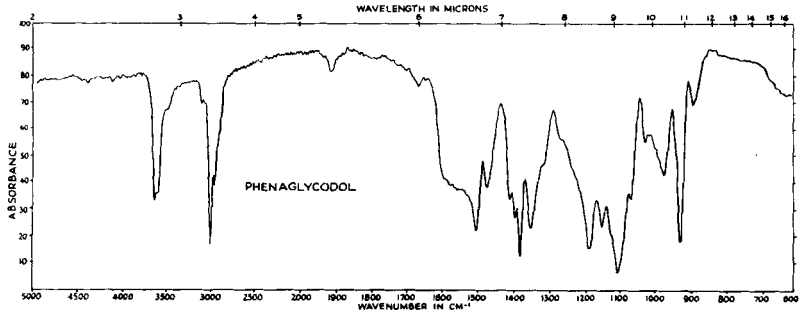

Fig. 2—Infrared absorption spectrum of phenaglycodol, 10 mg./ml. in carbon tetrachloride.

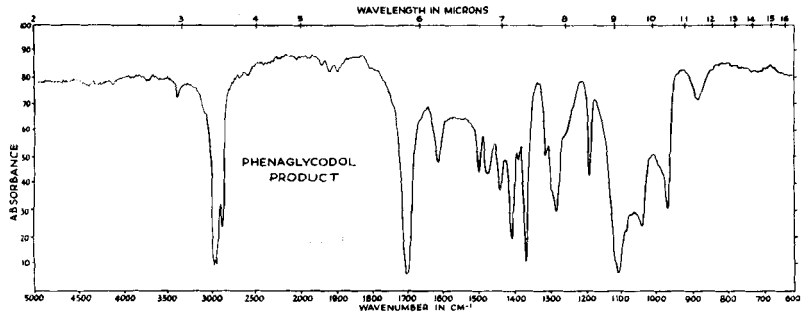

Fig. 3—Infrared absorption spectrum of the phenaglycodol reaction product, 10 mg./ml. in carbon tetrachloride.

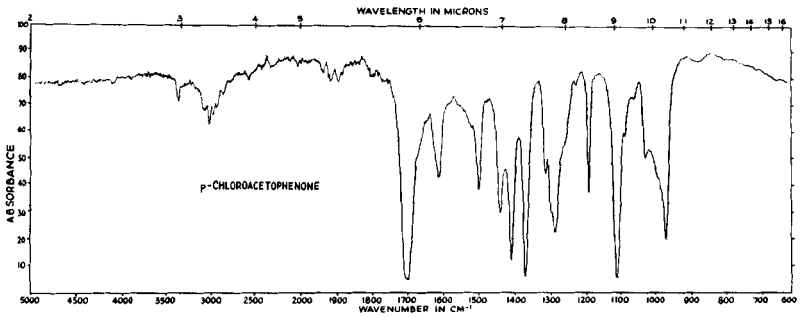

Fig. 4—Infrared absorption of p-*chloroacetophenone, 10 mg./ml. in carbon tetrachloride.*

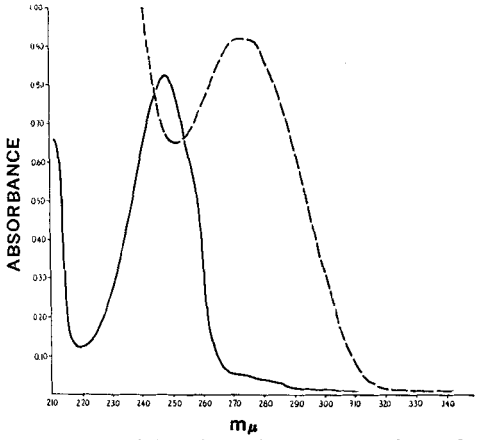

Fig. 5—Ultraviolet absorption spectra of p-*chloroacetophenone in hexane and of the semicarbazone of* p-*chloroacetophenone in water, each corresponding to a* p-*chloroacetophenone concentration of 7 mcg./ml. Key: ——, p-chloroacetophenone; ---, semicarbazone of* p-*chloroacetophenone.*

TABLE II—COMPOUNDS INVESTIGATED FOR INTERFERENCE WITH THE DETERMINATION OF PHENAGLYCODOL[a]

Compd.	Absorbance of Product[b]	Absorbance of Product Semicarbazone[c]
Phenaglycodol	1.69	1.90
Blank	0.01	0.01
Bisacodyl	0.05	0.01
Caffeine	0.01	0.01
Glutethimide	0.04	0.01
Hydroxyphenamate	0.05	0.08
Isonicotinic acid hydrazide	0.02	0.01
Isopropyl meprobamate	0.01	0.01
Meprobamate	0.01	0.01
Methyprylon	0.02	0.01
Phenacemide	0.04	0.03
Phenacetin	0.15	0.04
Phenol	0.03	0.01
Trimethadione	0.06	0.01

[a] Each compound was determined from whole blood. The level corresponds to a concentration of 20 mcg. of the compound per ml. of hexane. Each value is the average of three determinations. [b] Read at 247 mμ. [c] Read at 273 mμ.

glycol in the oxidation process is further substantiated by the lack of significant hydroxyl absorption at 3600 cm.$^{-1}$ in the spectrum of the product.

With the exception of strong carbon-hydrogen vibrations exhibited in the spectrum of the phenaglycodol product, the infrared spectra of the product and *p*-chloroacetophenone (Fig. 4) are very similar.

TABLE III—RECOVERY STUDIES OF PHENAGLYCODOL

Phenaglycodol Added, mcg./ml.	No. Determinations	Recovery,[a] Mean ± S.D. mcg./ml. Whole Blood	Urine[b]
50	12	45.5 ± 1.1	46.2 ± 1.7
25	10	23.7 ± 1.2	23.8 ± 1.3
10	9	9.7 ± 0.3	9.6 ± 0.2
5	11	4.6 ± 0.3	5.0 ± 0.2
Av. Recovery		93.7%	95.9%

[a] Determined from semicarbazone of reaction product. [b] Hydrolyzed prior to analysis.

TABLE IV—PHENAGLYCODOL LEVELS IN THE RAT[a]

Time, hr.	Phenaglycodol Recovered,[b] mcg./ml. or Gm. Blood	Brain	Kidney	Liver	Muscle
3	11.3	36.8	29.4	43.2	22.9
6	19.9	58.7	46.9	77.2	39.3
12	21.6	62.6	60.9	79.2	38.4
24	2.5	8.0	5.3	10.4	5.8

[a] Rats were given 104 mg. of phenaglycodol per Kg. body wt. [b] Determined from semicarbazone of reaction product.

TABLE V—PHENAGLYCODOL[a] DISTRIBUTION IN THE RAT

Tissue	Distribution Ratio,[b] Mean ± S.D. Concn. in Tissue/Concn. in Blood
Blood	1.0
Brain	3.2 ± 0.4
Kidney	2.6 ± 0.5
Liver	3.9 ± 0.3
Muscle	2.0 ± 0.4

[a] Unconjugated phenaglycodol. [b] Average of 15 rats.

TABLE VI—CONCENTRATION OF PHENAGLYCODOL[a] IN HUMAN BLOOD

Hours After Ingestion	Phenaglycodol Concentration,[b] mcg./ml. whole blood Adult I	Adult II	Adult III	Adult IV
0	0.0	0.0	0.0	0.0
0.5	0.5	0.4	...[c]	...
1	6.1	1.7	2.9	7.7
2	6.5	3.8	6.8	8.4
3	5.8	6.1	5.7	7.0
4	5.3	5.2	4.3	6.5
6	4.1	4.0	3.3	5.6
8	3.7	3.2	3.1	5.1
12	2.9	2.4	2.6	4.3
24	2.1	1.6	1.9	2.8

[a] Unconjugated phenaglycodol. [b] Each subject ingested a single 800-mg. dose after 12 hr. of fasting. [c] Not determined.

Ultraviolet spectral curves of the two are identical (Figs. 1 and 5) and gas-chromatographic retention data suggest that they are the same compound. Calculations utilizing the ultraviolet spectra of the phenaglycodol product and of *p*-chloroacetophenone

TABLE VII—PHENAGLYCODOL IN URINE AFTER INGESTION OF 800 MG. PHENAGLYCODOL EXCRETED[a]

Hr. After Ingestion	Adult I		Adult II		Adult III		Adult IV	
	Conjugated	Unconjugated	Conjugated	Unconjugated	Conjugated	Unconjugated	Conjugated	Unconjugated
4	30.2	0.49	32.2	0.29	19.9	0.38	23.7	0.51
8	36.1	0.19	16.3	0.23	15.0	0.16	27.4	0.47
12	22.5	0.11	42.8	0.14	8.4	0.04	32.9	0.46
24	57.6	0.44	42.0	0.11	42.2	0.30	60.1	0.56
48	69.0	0.56	60.3	0.07	40.6	0.36	83.2	0.93
72	32.9	0.24	35.4	0.06	32.6	0.51	30.2	0.29
96	13.1	0.26	15.1	0.03	12.5	0.22	13.7	0.19
120	6.3	0.34	6.3	0.02	7.5	0.19	4.3	0.14
Total	267.7	2.63	250.4	0.95	178.6	2.16	275.5	3.55

[a] Determined from semicarbazone of reaction product.

(Fig. 5) indicate that approximately 100% of the phenaglycodol is converted to *p*-chloroacetophenone.

Several drugs were investigated for interference with the determination of phenaglycodol. None produced a reaction product or semicarbazone which absorbed significantly throughout the range 220–360 mμ. The results of the study for interfering substances are presented in Table II. Standard alcoholic solutions of phenaglycodol were added to biologic material to provide concentrations from 0.50 to 5.0 mg. %. Ten milliliters of blood or hydrolyzed urine with added drug was then examined by the above method. Since phenaglycodol is predominantly conjugated in urine specimens (Table VII), hydrolysis provides a better appraisal of the method's ability to recover the drug from that specimen. Average recovery from blood was approximately 94% and from urine 96% (Table III).

To determine the tissue distribution pattern of the drug, rats were fed, *via* stomach tube, 104 mg. of phenaglycodol per kilogram of body weight. Blood and tissue levels after 3, 6, 12, and 24 hr. are shown in Table IV. Levels of the drug found in blood and tissues are given in decreasing order: liver, brain, kidney, muscle, and blood. Average distribution ratios for 15 rats are given in Table V.

Each of four adult human males who had fasted for 12 hr. received 800 mg. of phenaglycodol by mouth. Thereafter, phenaglycodol concentrations were determined in venous blood specimens drawn at predetermined intervals. The highest concentration of drug in the blood occurred 2–3 hr. after ingestion (Table VI). Phenaglycodol in the urine was predominantly excreted in the conjugated form (Table VII). Urine analysis without prior hydrolysis of the conjugated derivative indicates that less than 0.5% of the administered drug is eliminated unchanged in man during the first 120 hr. Five to 8% of the drug, however, was found in the urine during the first 24 hr. as the conjugated form. The concentration of conjugated drug measured in the urine over a 120-hr. period varied from one subject to another, but the total amount excreted in the urine by the four subjects was less variable: 178.6–275.5 mg. Cima and Fassina (4) found in their study with rabbits that in 4 days about 3% of the unchanged drug is excreted in the urine and an additional 31% is found as conjugated products, one of which they claim to be a glucuronide. Their report gives no tissue or blood levels of the drug.

The method described distinguishes between phenaglycodol and its conjugated metabolites, and affords the only available spectrophotometric analysis of the drug in blood and tissues. The sensitivity of the method permits the analyst to detect the drug in blood and urine after an individual has taken a therapeutic amount of the drug. The drug and its metabolites can be detected in the urine of adult humans for at least 5 days after the ingestion of a single 800-mg. dose.

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Keyphrases

Phenaglycodol
Body fluids, tissue—phenaglycodol determination
Semicarbazone, phenaglycodol—analysis
UV spectrophotometry—analysis
IR spectrophotometry—analysis