Secondary Standard for the Analysis of Glyceryl Trinitrate by Infrared Spectrophotometry

By ALMA L. HAYDEN

A method has been developed for the determination of glyceryl trinitrate by infrared analysis using benzoic acid as a secondary standard. The results of analyses of an adsorbate and of tablets agreed within 1.0 per cent with results obtained by the U.S.P. assay procedure and within 3.0 per cent with results obtained by a phenoldisulfonic acid colorimetric procedure.

THE U.S.P. assay procedure for glyceryl trinitrate tablets by infrared spectrophotometry (1) is based on the use of a reference standard supplied as an adsorbate on lactose. The concentration of the nitrate ester in the adsorbate is determined by the U.S.P. nitrate reduction method (2). Because of the unusual difficulties encountered in preparing and standardizing a stable reference standard for this compound, a search was made for a compound which would substitute for glyceryl trinitrate as a standard in infrared analyses.

It was anticipated that a reproducible ratio of infrared absorptivity coefficients for carbon disulfide solutions of glyceryl trinitrate and of a secondary standard would be found. From this ratio, the concentration of glyceryl trinitrate in adsorbates and in pharmaceutical preparations could be determined. Recently, Comer and Ribley (3) reported the successful use of benzoic acid as an absorbance standard in the analyses of various pharmaceuticals.

In the work reported here atropine, ethyl nitrate, *m*-dinitrobenzene, acetanilid, and benzoic acid were investigated as possible secondary standards. The concentration of the glyceryl trinitrate in the reference standard adsorbate was determined by the U.S.P. nitrate reduction method (2). The infrared measurements were made on carbon disulfide extracts of the adsorbate and on direct solutions of the secondary standards. Only for benzoic acid was the ratio of absorptivities ($2.04 \pm 1.8\%$) proportional to concentration. Because of its reproducible ratio value and its availability in high purity, benzoic acid was chosen as the secondary standard in determinations of glyceryl trinitrate in adsorbates and tablets.

EXPERIMENTAL

Preliminary Search for a Secondary Standard.—The concentration of glyceryl trinitrate in an earlier U.S.P. reference standard adsorbate was determined to be 9.26% by the U.S.P. nitrate reduction method (2). Subsequently, a qualitative infrared spectrum of glyceryl trinitrate was obtained by extracting 40 mg. of the adsorbate with 2 ml. of carbon disulfide and measuring the absorbance of the filtered extract. The bands at 5.99 and 7.88 μ were chosen for the quantitative infrared study. The baseline absorbance measurements of these bands revealed conformity to Beer's law, and recoveries were reproducible within $\pm 1.0\%$ over the concentration range of 0.1 to 0.7 mg. per ml. of carbon disulfide.

From qualitative infrared spectra, analytical absorption bands were chosen for atropine at 9.68 μ, ethyl nitrate at 7.82 μ, *m*-dinitrobenzene at 6.47 μ, acetanilid at 5.84 μ, and for benzoic acid at 5.89 and 7.79 μ. The absorptivity coefficient ($K = A/cd$) for each compound was determined at the selected analytical wavelength over the concentration range of 0.1 to 1.0 mg. per milliliter. The ratios of the absorptivity coefficients ($R = K_{Gt}/K_S$) of glyceryl trinitrate (G_t) to each of the secondary standards (S) were determined. The areas of the absorption bands for the secondary standards and for glyceryl trinitrate were determined from $(A \times H)/cd = K_H$, where H is the band width at one-half the height of the baseline absorbance peak. From these values, the ratio of K_{HGT}/K_{HS} (R_H) was determined for some of the compounds.

The absorbance measurements and ratio determinations were made over a period of months on solutions of different concentrations in cells 1.05 and 1.25 mm. thick on Perkin-Elmer model 21 and 237 infrared spectrophotometers. Quantitative instrument settings were used (at 5.5 μ on model 21, resolution = 984, slit = 82.5 μ, gain = 4.0; on model 237, slit = 80μ, gain set at no overshoot).

Method for Determination of Glyceryl Trinitrate in the Present U.S.P. Reference Standard and in Tablets Using Benzoic Acid as a Secondary Standard.—A portion of finely powdered sample equivalent to 1.5 mg. of glyceryl trinitrate was transferred to a 10-ml. glass-stoppered conical flask. Exactly 5.0 ml. of carbon disulfide was added, and the suspension was swirled gently for 30 seconds at 10-minute intervals over a period of 30 minutes. In the meantime, a solution was prepared of benzoic acid (freshly dried at 105° for 2 hours) in carbon disulfide at a concentration of 0.50 mg. per ml.

The sample suspension was allowed to settle completely. To prevent evaporation of the solvent during the filtration, an aliquot of the carbon disulfide sample layer was filtered through a pledget of cotton located in the needle of a syringe. The filtrate was transferred directly to a 1.0-mm. sodium chloride cell, and the quantitative absorbance spectrum of the region between 5.5 and 6.5 μ was recorded with carbon disulfide as the blank. Quantitative measurements of the benzoic acid solution were made over the same range with carbon disulfide as the blank. Baseline absorbance readings were made of the maxima near 5.9 μ using minima near 5.6 μ.

The quantity of glyceryl trinitrate in the adsorbate or tablets was calculated from

$$(A_B/C_B) \times 2.04 = K_{GT}$$

$$(A_{GT}/K_{GT}) \times 5.0 \times T/W = \text{mg./tablet}$$

where 2.04 is the ratio of the absorptivity coefficients

Received April 3, 1964, from the Division of Pharmaceutical Chemistry, Bureau of Scientific Research, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.
Accepted for publication October 14, 1964.

TABLE I.—RATIOS OF ABSORPTIVITY COEFFICIENTS[a]

Compd.	Band, μ	K	R	K_H[b]	R_H[c]
Glyceryl trinitrate	7.88	0.54	3.3 ± 2.2 %	...	...
Atropine	9.68	0.16	...	...	...
Glyceryl trinitrate	7.88	0.57	0.77 ± 3.8 %	...	...
Ethyl nitrate	7.82	0.74	...	...	...
Glyceryl trinitrate	5.99	1.20	Variable	5.07	3.75 ± 0.3 %
Acetanilid	5.84	0.39–0.45	...	1.35	...
Glyceryl trinitrate	5.99	1.23	1.85 ± 4.8 %	0.46	2.10 ± 2.0 %
m-Dinitrobenzene	6.47	0.67	...	0.22	...
Glyceryl trinitrate	5.99	1.28	2.04 ± 1.8 %	5.62	2.05 ± 0.4 %
Benzoic acid	5.89	0.62	...	2.74	...
Glyceryl trinitrate	7.88	0.55	1.60 ± 1.2 %	3.02	1.26 ± 0.6 %
Benzoic acid	7.79	0.34	...	2.89	...

[a] These ratio values (R and R_H) were determined with the earlier U.S.P. reference standard glyceryl trinitrate. [b] K_H = Absorptivity coefficient times half-band width in millimeters. [c] R_H = Ratio of K_H values.

TABLE II.—GLYCERYL TRINITRATE–BENZOIC ACID RATIO VALUES[a]

Expt. No.	Concn., mg./ml. GT[b]	B[b]	Bands, μ GT	B	Conditions: I.R. Spectrophotometer	Cell Thickness, mm.	R	R_H[c]
A	0.16	0.15	7.88	7.79	Model 21	1.05	1.62	...
B	0.31	0.73	7.88	7.79	Model 21	1.05	1.61	...
C	0.85	1.86	7.88	7.79	Model 21	1.05	1.61	...
D	0.29	0.59	7.88	7.79	Model 21	1.25	1.60	1.26
E	0.29	0.59	7.88	7.79	Model 237	1.25	1.77	1.24
F	0.35	0.70	7.88	7.79	Model 237	1.05	1.73	1.27
G	0.37	1.13	7.88	7.79	Model 237	1.05	1.75	1.23
H	0.15	0.62	5.98	5.89	Model 237	1.05	2.01	2.01
I	0.29	0.58	5.98	5.89	Model 21	1.05	2.04	2.05

[a] These ratio values were determined with the earlier U.S.P. reference standard. [b] Glyceryl trinitrate = GT; benzoic acid = B. [c] R_H = Ratio of K_H values.

TABLE III.—RESULTS OF ANALYSES OF PRESENT U.S.P. GLYCERYL TRINITRATE REFERENCE STANDARD

Procedure	% Glyceryl Trinitrate in Adsorbate
Method proposed	9.50, 9.38, 9.40 9.24, 9.40, 9.37 9.54, 9.36, 9.49 9.40, 9.50, 9.40 Av. = 9.42 ± 0.06
U.S.P. nitrate reduction	9.34 9.27 9.38 Av. = 9.34 ± 0.02
Colorimetric	9.37 9.29 9.45 Av. = 9.37 ± 0.05
U.S.P. infrared spectrophotometric	9.37 9.42 Av. = 9.40 ± 0.02

TABLE IV.—RESULTS OF ANALYSES OF GLYCERYL TRINITRATE PREPARATIONS

Sample	Description	Declared, mg.	Recovery, %—Proposed Method	Recovery, %—Other[a]
1	Tablets, 1/100 gr.	0.65	102.5	98.2
			102.3	99.4
			102.3	98.6
			101.7	100.0
2	Tablets, 1/400 gr.	0.16	100.6	101.3
			101.2	100.0
			...	96.9

[a] Results from the colorimetric procedure.

of glyceryl trinitrate and benzoic acid; A_B and A_{GT} represent the baseline absorbance values for the benzoic acid standard at the maximum at about 5.89 μ and for the glyceryl trinitrate sample at the maximum at about 5.99 μ; C_B is the concentration of the benzoic acid solution in milligrams per milliliter; T represents the average weight per tablet or 100 mg. of adsorbate; and W is the sample weight.

The identity of the sample was proved by concentrating a 4-ml. aliquot of the quantitative carbon disulfide solution to 1 ml. at about 40°, obtaining the infrared spectrum of the concentrate between 2 and 15 μ, and comparing to the spectrum of an authentic sample.

RESULTS AND DISCUSSIONS

Table I gives the ratios of absorptivities and band areas of glyceryl trinitrate and the secondary standards at the selected wavelengths. A ratio of 0.77 ± 3.8% was found for ethyl nitrate; however, on standing, the compound hydrolyzed slowly to ethyl alcohol. Therefore, the ratio of absorptivities varied over a period of months. The difficulty in maintaining pure stable secondary standards and the need for band area measurements influenced the elimination of atropine, *m*-dinitrobenzene, and acetanilid as secondary standards.

Benzoic acid was chosen as the secondary standard because it is available in high purity and because the ratio values are reproducible over a range of experimental conditions (Table II). Comparable instrument settings gave a constant R value. The constant R value of 2.04 and a freshly prepared benzoic acid solution were used in making deter-

minations of glyceryl trinitrate in the present U.S.P. adsorbate and in tablets. The results, given in Table III, agreed within 1.0% of the averages of results obtained by the nitrate reduction, infrared, and colorimetric methods. In general, the recoveries of glyceryl trinitrate from the tablets agreed within 3% of the amounts determined by the phenoldisulfonic acid colorimetric procedure (4), as given in Table IV.

In infrared spectrophotometry, it is preferable to compare a sample directly with the reference standard. However, secondary standards are useful when the reference standard is unstable, difficult to obtain or maintain, or otherwise presents a problem. This present use of benzoic acid in analyses of glyceryl trinitrate is one example of the application of a secondary standard in quantitative analyses.

The method is rapid and allows final identification of the analyzed material by its infrared spectrum.

SUMMARY

A method is presented for the infrared analysis of glyceryl trinitrate using benzoic acid as a secondary standard. In general, the results of analyses agreed favorably with those obtained by the U.S.P. nitrate reduction, by an infrared spectrophotometric method using a glyceryl trinitrate adsorbate standard, and by a phenoldisulfonic acid method.

REFERENCES

(1) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1st Suppl., 1962, p. 19.

(2) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960, p. 311.

(3) Comer, J. P., and Ribley, A. M., THIS JOURNAL, 52, 358(1963).

(4) Hohmann, J., and Levine, J., private communication.

Promazine Hyperthermia in Young Albino Mice

By WALTER J. BAGDON and DAVID E. MANN, JR.

The intraperitoneal administration of promazine hydrochloride to albino mice at a dosage level of 1 mg./Kg. of body weight caused hyperthermia in 10-day-old and hypothermia in 38-day-old animals. The removal of the chlorine atom from the structure of chlorpromazine decreased significantly the hyperthermic and hypothermic responses of the phenothiazine in immature and mature mice, respectively. No significant difference due to sex was noted in the activity of the drug at different ages.

DURING THE pioneer pharmacologic investigation of chlorpromazine, Courvoisier and her coworkers (1) observed that the phenothiazine caused a profound fall in body temperature in mature animals, a response that has been confirmed since in a wide variety of species, including man (1–5). Recently, this laboratory demonstrated that the effect of chlorpromazine on the body temperature of mice was influenced by age, for the drug elicited a significant hyperthermia in 10-day-old and hypothermia in 35- and 38-day-old animals (6).

Promazine, the dechlorinated analog of chlorpromazine, is qualitatively similar to the parent compound in its biological behavior, although quantitatively it is generally assumed to be less potent. Because many phenothiazines including promazine, are known to induce hypothermia in adult mammals, the purpose of this study was to ascertain whether the effects of promazine in immature mice were also influenced by age and, if so, to what extent.

EXPERIMENTAL

Albino mice (1140) (Huntingdon Farms, Inc., HTF strain) were divided into six groups, each containing from 119 to 238 animals, according to the following ages: 10, 15, 20, 25, 30, and 38 days. The age difference of mice within a group did not vary more than 15 hours. Except for the youngest age groups (10 and 15 days), each group was equally divided regarding sex. Food was withdrawn 15 minutes and water 1 hour before experimentation to provide for nutritional constancy. Immediately after weighing each mouse on a triple-beam Ohaus balance, the animal was confined in a 100-ml. glass beaker with a screen top and kept at a constant environmental temperature of 38° 15 minutes before injection and throughout the 30-minute observation period. Temperature recordings were taken orally to the nearest 0.1° immediately prior to and 30 minutes after each injection with a model 43 Tele-Thermometer equipped with a No. 402 probe.[1] Freshly prepared aqueous solutions of promazine hydrochloride[2] were administered at a dosage level of 1 mg./Kg. to one-half of a group (equally divided regarding sex), while the remaining mice received distilled water[3] and served as controls. All injections were intraperitoneal at a fixed volume of 0.1 ml.

The significance of difference between the means of drug and control temperatures was estimated by the *t* test, and probability levels were also indicated. Probability levels below 95% were designated insignificant.

RESULTS AND DISCUSSION

The effect of promazine hydrochloride, administered intraperitoneally at a dosage level of 1 mg./Kg., on the body temperature of young albino mice, was influenced by age (Table I). Table I shows that the differences between drug and control groups are significant at extremes of age only, and that there is no evidence that the differences in ages 20 through 30 days could not have occurred by chance. In two-hundred and eleven 10-day-old mice of undifferentiated sex, promazine produced an average increase in the oral temperature of approximately

Received June 22, 1964, from the Department of Pharmacology, School of Pharmacy, Temple University, Philadelphia, Pa.

Accepted for publication September 1, 1964.

Abstracted from a thesis submitted by Walter J. Bagdon to the Graduate School, Temple University, Philadelphia, Pa., in partial fulfillment of Doctor of Philosophy degree requirements.

[1] Yellow Springs Instrument Co., Yellow Springs, Ohio.

[2] Marketed as Sparine by Wyeth Laboratories, Philadelphia, Pa.

[3] Marketed as Water for Injection by the Philadelphia Ampoule Laboratories, Philadelphia, Pa.