Determination of Trace Amounts of Glycidol in Milk of Magnesia

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Abstract □ A very sensitive procedure is described to determine traces of glycidol in milk of magnesia formulated with 1% of this sterilant. Glycidol is separated from a milk of magnesia–diatomaceous earth column by continuous extraction with 1,2-dichloroethane and reacted with 2,4-dinitrobenzenesulfonic acid under anhydrous conditions. The resulting ester gives a yellow color with piperazine which is measured in a spectrophotometer. Even traces of water present in the reaction mixture interfere with esterification. Water is eliminated completely by the addition of 2,2-dimethoxypropane. In the presence of 2,4-dinitrobenzenesulfonic acid, it reacts with water to form methanol and acetone. No glycidol was found in milk of magnesia samples after 14 weeks of storage at room temperature. Quantitative recoveries were made for 1–27 p.p.m. glycidol added to milk of magnesia.

Keyphrases □ Milk of magnesia formulations—separation, analysis, glycidol sterilant □ Glycidol sterilant—separation, analysis, milk of magnesia formulations □ Colorimetry—analysis, glycidol in milk of magnesia formulations

A method for sterilizing aqueous pharmaceutical formulations with glycidol (2,3-epoxy-1-propanol) was described by Riffkin and Marcus (1). Because of the toxicity of this sterilant, a very sensitive procedure for determining traces of glycidol was needed. Such a procedure has been developed to monitor milk of magnesia formulated with 1% glycidol.

The colorimetric method described by Urbanski and Kainz (2) was selected for investigation because it appeared to be sensitive, simple, and selective. Glycidol reacts with 2,4-dinitrobenzenesulfonic acid under anhydrous conditions. When the resulting dinitrobenzenesulfonic ester is reacted with piperazine, it gives a yellow color which is measured spectrophotometrically. Even traces of water present in the reaction mixture interfere with the esterification of glycidol. Urbanski (3) also showed that the color intensity depended strongly on the water content. It was found in the present investigation that water can be eliminated completely during esterification by the addition of 2,2-dimethoxypropane. In the presence of acid, it reacts with water to form methanol and acetone (4). Glycidol is separated from milk of magnesia on a diatomaceous earth column by continuous extraction with 1,2-dichloroethane prior to its colorimetric analysis.

EXPERIMENTAL

Apparatus—Spectral measurements were made in a spectrophotometer[1]. The chromatographic column was 20 cm. long × 1.5 cm. i.d., equipped with a Teflon stopcock and a 200-ml. reservoir.

Reagents and Chemicals—Diatomaceous earth[2] (acid washed), 2,2-dimethoxypropane, anhydrous sodium sulfate, standard 0.1 *N* iodine, standard 0.1 *N* sodium thiosulfate, 0.25 *N* acetic acid, starch indicator solution[3], 0.5% 2,4-dinitrobenzenesulfonic acid in redistilled dioxane (prepared fresh daily), 2.5% piperazine in dimethylformamide, and redistilled and washed 1,2-dichloroethane were used. The dichloroethane is distilled at atmospheric pressure and washed successively with 1 *N* hydrochloric acid, 1 *N* sodium hydroxide, and distilled water until the final wash is neutral to phenolphthalein. The dichloroethane is washed the day it is used.

Preparation of Standard—*Assay*—Weigh about 60 mg. of glycidol into a 125-ml. conical flask. Pipet 25 ml. of standard 0.1 *N* sodium thiosulfate solution and 5.0 ml. of 0.25 *N* acetic acid into the flask and mix. Incubate the solution in an oven at 70° for 15 min. Cool the sample in an ice bath. Add 5 ml. of starch indicator solution, and titrate with standard 0.1 *N* iodine solution to a blue end-point. Calculate purity from the amount of thiosulfate consumed by the glycidol.

Dilution—Weigh about 60 mg. of glycidol of known purity into a 100-ml. volumetric flask. Dilute to volume with redistilled and freshly washed 1,2-dichloroethane.

Assay Procedure—*Separation*—Place a glass-wool plug at the bottom of the chromatographic column, and pack with 20 g. of anhydrous sodium sulfate. Accurately weigh about 5.5 g. of milk of magnesia into a 100-ml. beaker. Add 5 g. of diatomaceous earth and mix with a spatula. Transfer in portions to the column, gently compressing each time with a tamping rod.

Wash the beaker with 20 ml. of distilled and freshly washed dichloroethane and transfer to the column. Allow the solvent to pass by gravity into a 250-ml. separator. Add more distilled and freshly washed dichloroethane, and elute glycidol from the column by a positive pressure of about 2 psig. until 135 ml. of total eluate is collected.

Pipet 2 ml. of the diluted glycidol standard solution into a second 250-ml. separator containing 133 ml. of the distilled and freshly washed dichloroethane. Add 135 ml. of the dichloroethane to a third 250-ml. separator to serve as a blank.

Esterification—To each separator, add 5 ml. of 2,2-dimethoxypropane and mix. Add 25 ml. of 2,4-dinitrobenzenesulfonic acid solution and again mix. After 5 min., add 50 ml. of 1 *N* hydrochloric acid and shake for 1 min. Transfer the lower dichloroethane layer into another 250-ml. separator. Wash the acid aqueous layer with 25 ml. of dichloroethane for 30 sec., and transfer the lower layer into the separator containing the original dichloroethane solution. Wash the combined dichloroethane with 50 ml. of 2% sodium bicarbonate solution for 1 min.

Filter the bottom dichloroethane layer through anhydrous sodium sulfate into a 200-ml. volumetric flask. Wash the sodium bicarbonate solution with 25 ml. of dichloroethane and filter into the volumetric flask. Wash the sodium sulfate with dichloroethane, dilute to volume, and mix.

Color Development—Pipet 5 ml. of sample, 5 ml. of standard, and 5 ml. of the blank into 175-mm. test tubes. Pipet 2 ml. of the 2.5% piperazine solution into each tube and mix. After 10 min., measure the absorbance of the sample and standard in 1-cm. cells at the 390-nm. maximum with the blank in the reference cell.

If the sample absorbance reads over 1.0, dilute aliquots of both the sample and the blank from the 200-ml. flasks with dichloroethane. If the reading is low or if no reading is obtained, transfer the sample and blank solutions to 500-ml. round-bottom flasks and evaporate to dryness. Use a rotating vacuum evaporator and a water bath with a temperature up to 70° to speed the evaporation. Add dichloroethane to produce a concentration of about 3 mcg. glycidol/ml.

React 5.0-ml. aliquots of both blanks (diluted or concentrated and undiluted), the adjusted sample, and the standard with 2.0 ml. of the piperazine solution. After 10 min., measure the absorbance of

[1] Beckman DU.
[2] Celite 545.
[3] Fisher.

Table I—Effect of Piperazine Concentration on Color Intensity

Piperazine Concentration in Dimethylformamide, %	Absorbance
5	0.980
4	0.920
3	0.800
2.5	0.800
1	0.310
0.5	0.055

Table II—Elution of Glycidol from Diatomaceous Earth (5.5 g. of Milk of Magnesia plus 5.0 g. of Diatomaceous Earth)

25-ml. Dichloroethane Fractions	Glycidol Eluted, %
1	39
2	26
3	17
4	11
5	7
6	0

these four solutions *versus* dichloroethane in the reference cell. Subtract the absorbance of the adjusted blank from that of the adjusted sample and the reading of the undiluted blank from the standard reading.

Calculate the quantity (micrograms of glycidol per gram of milk of magnesia) by the formula $(A_u - B_u/A_s - B_s)(W_s \times P/W_u)(V_F/V_I)20$, where A_u and A_s are the absorbances of the sample and standard solutions, respectively, and B_u and B_s are the absorbances of their respective blanks (if the sample was diluted or concentrated); W_s is the weight, in milligrams of the standard glycidol, and P is the purity of the standard (%/100); W_u is the weight, in grams of the milk of magnesia sample; V_F is the final volume, in milliliters of the diluted or concentrated sample; and V_I is the volume of sample, in milliliters taken initially for dilution or concentration.

RESULTS AND DISCUSSION

Glycidol Purity—Leary's (5) procedure for determining oxiranes with sodium thiosulfate was investigated for assaying the purity of glycidol. Reaction between glycidol and thiosulfate is quantitative under the conditions described in the procedure under *Preparation of Standard*. To verify this, glycidol was purified by solvent extraction from an aqueous solution into ether (6). The purity of this sample was 99.0% or greater, with a two- or threefold equivalent excess of sodium thiosulfate to glycidol. With a 1.5-fold excess of thiosulfate, only 95% of the glycidol reacted. Reaction of glycidol and thiosulfate was dependent on both time and temperature. Samples were heated in a 70° oven for 10, 15, and 30 min.; 15 min. was needed for completion of the reaction. Four replicate assays of one glycidol sample, using the recommended procedure, gave results varying from 91.0 to 91.4%.

Chemistry of Method—Reaction of glycidol with 2,4-dinitrobenzenesulfonic acid to yield an ester proceeds in dioxane, methylene chloride, and chloroform, as well as dichloroethane. Very little ester is formed in ether or carbon tetrachloride. The reaction does not proceed in butanol.

Table III—Recovery of Glycidol from Milk of Magnesia

Added Glycidol, mcg.[a]	Dichloroethane Final Volume, ml.	Absorbance	Recovery, %
146.2	200	0.940	99.5
97.4	200	0.624	99.1
48.7	200	0.320	101.6
24.4	200	0.156	99.2
4.87	20	0.324	102.8
0	20	0	—
Standard (97.4)	200	0.630	—

[a] Added to 5.5 g. of milk of magnesia.

Table IV—Decomposition of Glycidol in Milk of Magnesia

Storage, days	Glycidol, mg./g. Room Temperature (about 25°)	Glycidol, mg./g. 40°
Initial	9.91	10.02
1	8.56	8.54
4	7.00	4.88
7	5.92	2.37
14	4.68	0.62
21	3.27	0.20
28	2.13	0.034
35	1.62	0.000
42	1.08	0.000
49	0.74	—
56	0.58	—
63	0.44	—
70	0.27	—
77	0.153	—
84	0.024	—
93	0.000	—

Even traces of water interfere with the esterification. Drying dichloroethane extracts of glycidol with anhydrous sodium sulfate, calcium chloride, or magnesium sulfate before adding the dinitrobenzenesulfonic acid gave slightly higher absorbance values than those for undried solutions. But results were low compared with 2,2-dimethoxypropane-treated samples and varied from run to run. Previous investigators (4) used hydrochloric acid to catalyze the reaction of water with dimethoxypropane. In this procedure, the 2,4-dinitrobenzenesulfonic acid serves as both catalyst and colorimetric reagent.

The concentration of 2,4-dinitrobenzenesulfonic acid is important. About 150 mcg. of glycidol was reacted with various concentrations of the reagent in dioxane. A maximum absorbance was found between 0.1 and 0.5%. The higher concentration of reagent was selected to compensate for its consumption by impurities in solvents or samples. Colored products formed from impurities encountered in this laboratory were removed from the dichloroethane solution by later acidic and basic washes.

Absorbance readings of an unwashed blank solution increase with time. Fortunately, the absorbance of the blank is nearly eliminated by successive washings with 1 *N* hydrochloric acid and 2% sodium bicarbonate solutions. The reaction product of glycidol and 2,4-dinitrobenzenesulfonic acid is not extracted with these washes. After the aqueous washes, sample volumes can be reduced to increase the sensitivity of the method. The reaction product is a nonvolatile solid, and its dichloroethane solutions are stable at room temperature for at least 1 month. Because of this stability, the standard and blank solutions can be kept at room temperature and used as needed.

The concentration of piperazine for color development is important. Color intensity increases with the concentration of piperazine, as shown in Table I. Because the intensity of color depends on the concentration of piperazine, the 2 ml. of piperazine solution is added by pipet in the *Color Development* step of the procedure.

Solutions of the reaction product of glycidol with 2,4-dinitrobenzenesulfonic acid and piperazine follow Beer's law. A solution containing 1 mcg. of glycidol/ml. had an absorbance reading of 0.142.

Separation from Milk of Magnesia—Glycidol can be extracted from milk of magnesia with methylene chloride, dichloroethane, chloroform, ether, and other solvents. About eight 25-ml. portions of these solvents are needed to extract glycidol completely from 5 ml. of milk of magnesia in a separator. Since this extraction is tedious, continuous elution of glycidol from a diatomaceous earth column is used. As shown in Table II, 125 ml. of dichloroethane is needed for complete elution of glycidol.

Recovery—Various amounts of glycidol were added to milk of magnesia and subsequently assayed. Results are summarized in Table III.

Stability—Samples of milk of magnesia were sterilized with 1% glycidol and stored at room temperature (about 25°) and 40°. The decrease in the glycidol concentration is shown in Table IV.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 15, 1972, from the *Squibb Institute for Medical Research, New Brunswick, NJ 08903*

Accepted for publication September 5, 1972.

Presented to the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, March 1971.

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PHARMACEUTICAL TECHNOLOGY

Mechanism of Action of Starch as a Tablet Disintegrant VI: Location and Structure of Starch in Tablets

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Abstract □ By using a scanning electron microscope, the location and structure of starch grains in experimental and commercial aspirin tablets and aspirin–phenacetin–caffeine tablets were studied. By scrutinizing tablet faces and cross sections before and after the addition of water, it was observed that rupture of the surfaces occurred where starch agglomerates were found. It was postulated that water hydrates the hydroxy groups of the starch molecules, causing them to move apart. The slight swelling that occurs is due to the rapid hydration step and a slower sorption of addition water step. Channels or pores lined with starch were not evident. The conditions for rapid tablet disintegration are sufficient starch agglomerates, low pressure, and presence of water.

Keyphrases □ Starch as tablet disintegrant—location, structure in aspirin and aspirin–phenacetin–caffeine tablets, scanning electron microscope □ Aspirin, aspirin–phenacetin–caffeine tablets—location and structure of starch, scanning electron microscope □ Tablets, aspirin, aspirin–phenacetin–caffeine—location and structure of starch, scanning electron microscope □ Scanning electron microscopy—location, structure of starch in aspirin and aspirin–phenacetin–caffeine tablets □ Disintegrants—mechanism of action of starch in aspirin and aspirin–phenacetin–caffeine tablets, location and structure of starch agglomerates, conditions for rapid disintegration

Previous study has shown that corn and waxy maise starch grains plastically deform and that this deformation increases with increasing pressure. The addition of water has no apparent effect on this deformation (1, 2). Potato starch grains also have been shown to deform (3). It was also previously observed that when a small amount of moisture was added to corn and waxy maise starch tablets, a "blistering" effect and disruption of the moistened area occurred. Moisture did not cause the individual grains to regain their shape (1).

It is not practical to attempt to measure a change in volume due to the addition of moisture by the scanning electron microscope, because sample preparation for the scanning electron microscope involves the use of a vacuum which would remove the moisture and the sample is coated with a thin layer of a conductor of carbon and gold. When conductor layering was compared to an electrostatic method of sample preparation, similar results were obtained so that the scanning electron photomicrographs show a true picture of the surfaces (4).

The purpose of this investigation is to show the location of and the structure formed by the starch grains in experimental and commercial aspirin tablets and aspirin–phenacetin–caffeine tablets. The effect of moisture on these tablets is also shown.

EXPERIMENTAL

Tablets weighing 0.5 g. were compressed using 1.27-cm. (0.5-in.) diameter flat-face punches and die. Aspirin[1] was sieved to obtain 40–50- and 70–100-mesh crystal fractions. These fractions were mixed with starch[2] to give 2.5, 5, 7.5, 10, 12.5, and 15% (w/w) starch concentrations. The ingredients were mixed by tumbling. Tablets were made by compression at 10,000 psig. on a hydraulic laboratory press[3] with special holders for the punches and die. An aspirin–10% starch granulation[4], 12–50 mesh, was also used. Tablets from the commercial granulation were made by compression at 2500 psig. At higher pressures the tablets appeared to be impervious to water and did not disintegrate. For the photomicrographs only, the 40–50-mesh aspirin with 2.5 and 10%

[1] Catalog No. A-42, lot 752285, Fisher Scientific Co.
[2] STR-Rx, A. E. Staley Manufacturing Co.
[3] Model B, Fred S. Carver, Inc., Hydraulic Equipment.
[4] Lot QM-18, Monsanto Chemical Co.