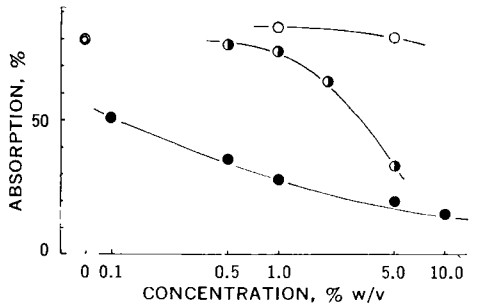

Figure 9—*Effect of sorbitan, polyoxyethylene 20 sorbitan, and polysorbate 80 on the absorption of isonicotinamide at the end of 3 min. Each point represents the mean value of at least five animals. Key: ⓪, control; ○, sorbitan; ◑, polyoxyethylene 20 sorbitan; and ●, polysorbate 80.*

tions suggest the possibility that the delayed absorption of a drug is caused by the surfactant *per se* and not by its hydrolysis products.

The results in this study indicate that: *(a)* a low concentration of the polysorbates dramatically reduces the absorption of water-soluble, micelle-free drugs and such reduction is reflected on the plasma levels of drugs; *(b)* the relative absorption rate of a drug solution in the muscle is dependent on the surfactant concentration rather than on the absolute amount; and *(c)* the delayed clearance seems to be operative in a variety of drugs and is caused by the polysorbates *per se*. The exact cause of the phenomenon is uncertain at present. Several mechanisms could operate alone or in combination to bring about the decrease of absorption observed in the presence of the polysorbates. Previous investigations concerning the absorption and viscosity of aqueous injection solutions (3) and on the membrane transport phenomena (16) in the presence of a surfactant under negligible micellar interaction ruled out the possibility that the reduction of the rate of absorption is caused mainly by the decreased diffusion rate of drugs in the injected solution containing surfactant. There is a possibility that the biological events that depend on other complex and interrelated events, which cannot be adequately explained by a simple physicochemical concept, influence the absorption.

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PHARMACEUTICAL ANALYSIS

GLC Analysis of Ergonovine Maleate

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Abstract □ A method is described for the quantitative determination of ergonovine maleate by GLC. Data are presented to show that this method provides the quantitative and semiquantitative results of the USP colorimetric assay and TLC in a single determination. Favorable quantitation is achieved by using brucine as internal standard. The sample is dried in the dark, derivatized with *N*-trimethylsilyldiethylamine and *N*-trimethylsilylimidazole in pyridine, and chromatographed on a column containing a nonpolar methyl silicone liquid phase. Degradation of the sample by preanalytical preparation (pH changes, air, heat, and light) are thereby minimized.

Keyphrases □ Ergonovine maleate—GLC analysis □ GLC—analysis, ergonovine maleate

It is generally recognized that the chemical purity of ergonovine (I) maleate is affected by oxygen, light, temperature, and extremes in pH (1–7). The chemical nature of the degradation products (6, 7) may cause falsely high values when assays are performed by the colorimetric method described in USP XVIII

Table I—Quantitative Analysis of Ergonovine Maleate by GLC

	Label	Range Found	*RSD*	*RE*[a]
Injectables[b]				
Ampuls Disposable syringe	0.2 mg/ml	0.198–0.202	±1.0%	−1%
Tablets[c]	0.2 mg/tablet	0.197–0.209	±3.0%	−2%

[a] Determined by the method of standard addition. [b] Ergotrate Maleate ampuls and disposable syringe, Lilly. [c] Ergotrate Maleate tablets, Lilly.

(8). Therefore, the sample must also be examined in a semiquantitative way by TLC to estimate the amount of degradation.

Analytical methods based on paper chromatography (9–11), TLC (12–14), gel permeation chromatography (15), ion-exchange chromatography (16), and thin-layer electrophoresis (17) have been proposed. These methods either are unable to resolve interferences or require excessive handling conducive to degradation during sample preparation.

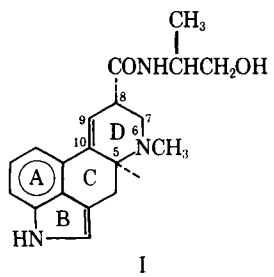
GLC has been used for the identification of narcotic drugs (18, 19) and alkaloids (20–28). A quantitative procedure for the measurement of lysergic acid diethylamide (lysergide) used ergonovine, which is lysergic acid 2-aminopropanolamide, as an internal standard (21). The method required catalytic hydrogenation of the two compounds prior to analysis and was found to be relatively insensitive and irreproducible (22). GLC of lysergic acid derivatives has been attempted, but only the amide and diethylamide produced unique peaks (28). Monosubstituted amides were pyrolyzed to the amide. Attempts to produce stable volatile derivatives were not successful.

This report describes the quantitative GLC analysis of ergonovine maleate using a trimethylsilyl derivative. The method provides the quantitative and semiquantitative results obtained by the two USP procedures in a single determination. Furthermore, evidence is presented that excessive handling, as by multistep extractions, causes degradation and premature rejection of pharmaceutically satisfactory material.

EXPERIMENTAL

Equipment—A gas chromatograph[1] equipped with a flame-ionization detector was used. The detector signal was fed to a computer[2] for peak integration and to a 1-mv recorder[3] with a chart speed of 38.1 cm (15 in.)/hr and a 1-sec full-scale response. Samples were injected with a 10-μl syringe[4].

Materials—Helium was used as a carrier gas, and electrolytic hydrogen and oxygen were used in the detector. The stationary phase was 1% OV-1 on Gas Chrom Q[5], 80–100 mesh, packed in borosilicate glass columns, 1.2 m × 0.64 cm o.d. All chemicals used were reagent grade or the best quality available.



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Operating Conditions—The column was operated isothermally at 260° with the detector block and injection port at 280°. The helium flow rate was 55 ml/min with an inlet pressure of 40 psi. The electrometer range was 10 with an attenuation of 16. Sample injections of 1 μl were made.

Preparation of Liquid Formulations—*Standard Solutions*—Solutions of ergonovine maleate reference standard, 0.2 mg/ml, were prepared by dissolving the solid in the internal standard solution. Standards were prepared daily and used immediately. One-milliliter portions were added by pipet to screw-cap vials, fitted with Teflon septa, and freeze dried.

Sample Solutions—One-milliliter aliquots, 0.2 mg/ml, of ergonovine maleate[6] were added by pipet to screw-cap vials. An equal volume of internal standard was added and the samples were freeze dried.

Internal Standard Solutions—Brucine was selected for use because of its known retention time under these GLC conditions (29) and its water solubility. Concentrations of 0.2 mg/ml were used.

Preparation of Tablet Formulations—*Standard Solutions*—Solutions of ergonovine maleate reference standard, 0.2 mg/ml, were prepared by dissolving the solid in pyridine–water (2:1), previously prepared and cooled to room temperature. One-milliliter portions were added by pipet to screw-cap vials, fitted with Teflon septa. Two milliliters of internal standard, in the same solvent, was added and the solution was evaporated under nitrogen without heat and protected from light.

Sample Solutions—A single tablet of ergonovine maleate[6] or its

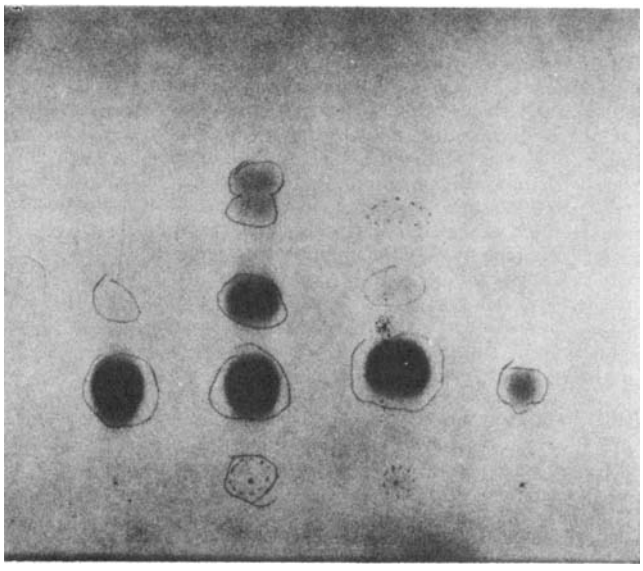

Figure 1—*TLC of ergonovine maleate performed in the system described in Ref. 8. Key (left to right): Lane 1, standard; Lane 2, sample extracted as per Ref. 8; Lane 3, sample lyophilized; and Lane 4, standard (3:100 dilution).*

[1] Hewlett-Packard, model 402.
[2] IBM 1800.
[3] Honeywell Electronik 16.
[4] Hamilton No. 701.
[5] Applied Science Laboratories, State College, Pa.
[6] Ergotrate Maleate (Eli Lilly and Co.), 0.2 mg/ml and 0.2 mg/tablet.

equivalent weight of a finely ground composite was placed in a centrifuge tube. Two milliliters of internal standard in pyridine–water solution was added by pipet. The tablet disintegrated readily and was intermittently agitated for 20 min. The solution was then centrifuged at 400 rpm for 5 min, and the clear supernate was decanted into screw-cap vials fitted with Teflon septa. The solvent was evaporated under nitrogen as previously described.

Internal Standard Solution—Pyridine–water (2:1), containing 0.1 mg/ml of brucine, was used.

Derivatization—Approximately 75 μl of *N*-trimethylsilyldiethylamine (II), 150 μl of dry pyridine, and 100 μl of *N*-trimethylsilylimidazole (III) were added to each vial through the Teflon septum using a tuberculin syringe. The liquid was then brought into contact with all of the glass surface inside the vial. Aliquots of 1 μl were taken for GLC.

Calculations—For each chromatogram, the ratio *(R)* of the area of the ergonovine peak to that of the internal standard was determined. Then the concentration of ergonovine maleate per milliliter of liquid formulations is given by:

$$\text{milligrams ergonovine maleate per milliliter} = \frac{R\ \text{sample}}{R\ \text{standard}} \times \frac{\text{milligrams standard}}{\text{milliliter standard solution}} \times \text{purity of standard} \quad \text{(Eq. 1)}$$

and:

$$\text{milligrams ergonovine maleate per tablet} = \frac{R\ \text{sample}}{R\ \text{standard}} \times \frac{\text{milligrams standard} \times \text{purity}}{\text{milliliter standard solution}} \times \frac{\text{average tablet weight}}{\text{milligram sample}} \quad \text{(Eq. 2)}$$

Colorimetric Analysis and TLC—Standards and samples were prepared and analyzed by the methods described in the USP (8).

RESULTS AND DISCUSSION

Although excellent reproducibility can be obtained with the colorimetric assay for ergonovine maleate (8), TLC repeatedly gives erratic results. To investigate this, two samples were taken. One was prepared by the USP method (8), and the other was freeze dried to eliminate the extraction procedure entirely. The latter sample was redissolved in 100 μl of ethanol and spotted for TLC along with the first sample and a standard (Fig. 1). The results clearly showed that sufficient degradation had occurred during the USP procedure to cause rejection of the material for pharmaceutical use. The freeze-dried sample showed no such effect. The USP procedure was repeated with modifications of pH, rapidity, temperature, solvent, exposure to light, and gaseous atmosphere. Each gave evidence of excessive degradation.

N,O-Bis(trimethylsilyl)acetamide was reported (30) to derivatize hydroxyl, indole, and perhaps amide groups. However, this reagent did not give a unique peak with ergonovine maleate on GLC. Compound III was reported to be a stronger reagent (31). This reagent and also II gave single peaks with ergonovine maleate. The latter reagent, however, dissolved the sample very slowly. Experimentation demonstrated that the most consistent results could be obtained using a mixture of the two reagents in pyridine as described under *Experimental*.

Degraded samples, either aged or extracted by the USP method, were treated with the silylating reagents and subjected to GLC (Fig. 2). Three peaks were clearly observed. Authentic samples of some identified degradation products (5–7) of ergonovine maleate were also subjected to derivatization and GLC. In this way, the two smaller peaks in the chromatogram can be tentatively identified as ergonovinine (peak A, Fig. 2) and lumiergonovine I (peak B, Fig. 2). Peak A was also obtained from the degradation spot observed on TLC. Ergonovinine is epimeric to ergonovine (I) at C-8. This position is made particularly sensitive to pH changes by its proximity to the carboxamide on one side and to the double bond on the other. Lumiergonovine I is the result of hydration of the 9,10-double bond with the hydroxyl in one of two possible configurations at C-10.

Attempts to obtain GLC–mass spectra of the observed peaks were unsuccessful. When the peaks were trapped and submitted to TLC, they had almost completely decomposed (presumably due to the heat and light at the column exit). The following is evidence for tentative identification of the observed peak as *N*(amide),*N*(indole),*O*-tris(trimethylsilyl)ergonovine. No lysergamide peak was observed in comparison to an authentic sample, derivatized and underivatized. Thus, the amide nitrogen is doubly substituted since underivatized monosubstituted lysergamides pyrolyze (28).

It is a certainty that the hydroxyl has been derivatized since both II and III are reactive toward alcohols (31). Treating a sample of ergonovine with II alone produced a peak with a shorter retention time than that produced by III. Compound III is reactive toward indole nitrogen (31). Therefore, the derivative with II is *N*-(amide),*O*-bis(trimethylsilyl)ergonovine and that with III is *N*-(amide),*N*(indole),*O*-tris(trimethylsilyl)ergonovine.

Solutions of differing ergonovine concentrations were prepared to establish the linearity of the procedure. The extrapolated results passed through the origin. Replicate standards and samples provided a residual standard deviation of ±1.0% and a relative error of less than 1% for injectables.

Tablets presented an additional problem of excipients. To separate ergonovine maleate from the solid, the tablet was suspended in 1 ml of aqueous internal standard and centrifuged and the

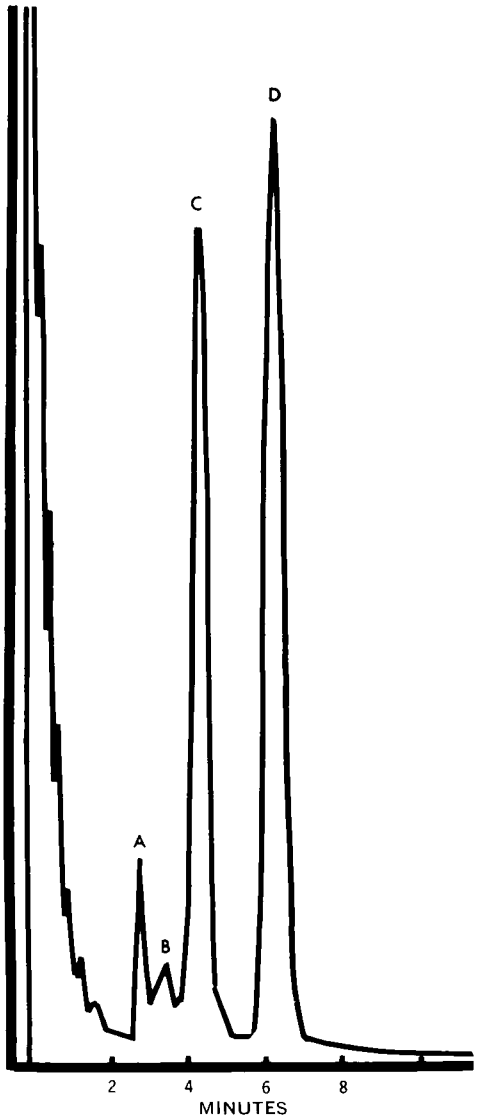

Figure 2—*GLC of derivatized ergonovine maleate (for conditions see text). Key: Peak A, derivatized ergonovinine; Peak B, derivatized lumiergonovine I; Peak C, derivatized ergonovine; and Peak D, brucine (internal standard).*

supernate was freeze dried. The residue was treated with silylating reagents but a portion remained insoluble. GLC of aliquots of the supernate did not give reproducible results. There was evidence of a large amount of degradation, thought to be due to the concentration of excipient potassium acid tartrate in the aqueous suspension. Alcohol–water mixtures were tried with poor results. Pyridine–water mixtures afforded the best results and the solution could be evaporated under nitrogen. There was little evidence of excessive degradation during sample workup, and the residue left after evaporation of the supernate was almost completely soluble in the silylating reagents. The small amount of insoluble material did not interfere with sampling for GLC. Replicate samples provided a residual standard deviation of ±3 and −2% relative error (Table I).

Some degradation always occurred during the sample workup. The sensitivity of the compound makes this unavoidable. As demonstrated in Fig. 1, however, the amount of degradation was small, and it is advisable to treat the samples and standard in the same way [unlike the USP method (8)] to compensate. This was also demonstrated by chromatography of derivatized reference standard weighed directly into the screw-cap vial and silylated. Peaks A and B (Fig. 2) were absent, while both were present in standards prepared and treated as the samples. The respective size of the peaks appeared to correlate with the age of the sample, peak A increasing faster than peak B, but this aspect was not pursued further.

This method should prove useful in the analysis of preparations of ergonovine and of methylergonovine which has a slightly longer retention time. The elimination of extraction procedures minimizes difficulties encountered due to degradation during analysis.

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