The reason for this difference is not readily apparent from the limited analytical data available. In addition, mean differences of 2.6 and 3.0% of the percent of the declared amount were obtained for the products containing thimerosal and phenylmercuric acetate, respectively.

The product containing phenylmercuric nitrate, which gave a mean value of 77.4% of the declared amount by the atomic absorption procedure, could not be analyzed by adaptation of official methodology due to the low concentration of this ingredient. Additional atomic absorption analysis of this product utilizing heating condition C gave a mean value of 78.5% of the declared amount. Approximately 13 months after the initial analyses were performed, the product was reassayed by both the proposed atomic absorption method and a total digestion procedure (19). The results obtained from single determinations were 60.7 and 60.8% of the declared amount, respectively. These observations provide corroborative evidence that adsorption of this compound by the container material (polyethylene in this case) may occur over an extended period (16). A simulated preparation of the product was formulated in this laboratory and subjected to atomic absorption analysis utilizing heating condition B. The mean recovery for the phenylmercuric nitrate based on duplicate results was 99.6% with a range of 0.8%.

An orange precipitate was formed during the protolysis of the merbromin reference standard, which, upon isolation and total digestion under reflux conditions (19), revealed the absence of mercury. The product containing merbromin showed a similar precipitate during this stage of the analysis.

Recovery experiments employing heating condition B (hotplate) with mercuric chloride standards representing quantities of mercury in the range encountered with the commercial preparations yielded values between 98.7 and 100.4%. This finding indicated that loss of mercury due to volatilization was negligible.

At the sensitivity level employed in the atomic absorption procedure, the use of the background correction mode indicated the absence of interferences due to nonspecific absorption.

In general, the results obtained indicate the overall applicability of the described atomic absorption procedure or modifications thereof to the analysis of various mercurial compounds present in bulk form, as reference compounds, or in pharmaceutical mixtures. The method also should be adaptable to products containing *o*-hydroxyphenylmercuric chloride and various other preparations containing thimerosal.

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GLC Determination of Hexadiphane in Pharmaceutical Preparations

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Abstract □ A specific, rapid, and sensitive GLC method for purity control of hexadiphane and its determination in pharmaceutical preparations is described. The method utilizes an extraction of the free base, followed by GLC on a 0.5% OV-17 column at isothermal temperature for 6 min and then the temperature was programmed. Results from this method and from a titrimetric method were compared, and no significant differences were found.

Keyphrases □ Hexadiphane—GLC analysis in pharmaceutical formulations □ GLC—analysis, hexadiphane in pharmaceutical formulations

Hexadiphane, 1,1-diphenyl-3-hexamethyleneiminopropane (I), is a papaverine-like compound with weak anticholinergic effects. It is widely employed as an antispasmodic (1–5).

Quantitative determination of hexadiphane is essentially based on the chemistry of the imine moiety. The methods employed are those used for basic nitrogen compounds, primarily titration in nonaqueous

Table I—Assay Results of the Liquid Commercial Preparation of Hexadiphane Hydrochloride (Label Claim, 2 mg/10 ml)

Analysis Number	Titrimetric Method		GLC Method	
	Found, mg	Recovered, %	Found, mg	Recovered, %
1	2.03	101.5	2.03	101.5
2	2.01	100.5	1.98	99.0
3	2.03	101.5	1.98	99.0
4	2.04	102.0	1.99	99.5
5	2.04	102.0	2.03	101.5
6	2.05	102.5	2.04	102.0
Mean	2.033		2.008	
SD	0.0136		0.0277	
RSD, %	0.669		1.379	
$F = 4.16$	$t = 1.96$			

solvents or with anionic surfactants and determination with picric acid (trinitrophenol), silicotungstic acid, tetraphenylboron, reineckate, and acid dyes (6). Such methods offer accuracy and precision and many are listed in the current pharmacopeias. However, they lack specificity and often require long and complicated separations.

Several GLC methods have been proposed for the determination of basic nitrogen compounds. Greenwood and Guppy (7) extensively reviewed the relevant literature. The GLC technique offers greater specificity, rapidity, and simplicity than do "classical" methods, with no loss of precision or accuracy.

The need for specificity and rapidity in the hexadiphane determination is great because the compound, which is obtained by decyanation of 1,1-diphenyl-3-hexamethyleneiminobutyronitrile (II), must be checked during the reaction to make sure that a quantitative reaction has occurred.

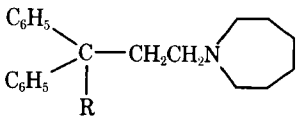
This paper describes a rapid quantitative GLC method for the determination of hexadiphane in pharmaceutical preparations. The preparations examined included a liquid form for oral use[1], a combination with oxazepam hemisuccinate in hard gelatin capsules[2], and a combination with digestive enzymes and dimethylpolysiloxane in coated tablets[3]. With the two solid forms, the analysis was carried out on specially prepared mixtures containing all components of the preparations.

The results were compared with those observed following titration with an anionic surfactant (0.01 *M* sodium lauryl sulfate).

EXPERIMENTAL

Equipment and Materials—The following were used: a gas chromatograph[4], dual column, equipped with flame-ionization detectors; a recorder[5]; a stainless steel column, 2 m × 2 mm i.d., packed with 0.5% methyl phenyl silicone (OV-17) on Gas Chrom Q, 80–100 mesh; a 10-μl syringe[6]; hexadiphane hydrochloride[7], hexadiphane maleate[7], and diphenylpyraline hydrochloride, pure to GLC analysis and at least 99.5% pure to titration in nonaqueous

[1] Commercially available.
[2] Spasmo Nulans Schiapparelli.
[3] Prandium Schiapparelli.
[4] Fractovap model D, C. Erba.
[5] Speedomax G, Leeds and Northrup Co.
[6] Hamilton.
[7] Synthesized in these laboratories.



I: R = H
II: R = CN

solvents; daily-distilled ether; carbon disulfide, analytical grade; ether-washed anhydrous sodium sulfate; 0.1 *N* hydrochloric acid; and 1 *N* sodium hydroxide.

Operating Conditions—The column was conditioned by heating at 250° for 1 hr with carrier gas flow, then at 300° for 4 hr without carrier gas flow, and finally at 250° for 18 hr with carrier gas flow.

The operational parameters were: column temperature, 190°, isothermally for 6 min and then programmed to 250° at a heating rate of 22°/min; injection port temperature, 300°; detector temperature, 280°; carrier gas, nitrogen at 35 ml/min; hydrogen, 25 ml/min; air, 0.5 kg/cm^2; and attenuation, 10 × 128.

Hexadiphane Standard Solution—About 25 mg of hexadiphane hydrochloride, accurately weighed, was dissolved in water and diluted to 100 ml in a volumetric flask.

Internal Standard Solution—About 16 mg of diphenylpyraline hydrochloride, accurately weighed, was dissolved in water and diluted to 100 ml in a volumetric flask.

Correction Factor and Calibration Curve—Two milliliters of the hexadiphane standard solution and 2 ml of the internal standard solution were pipetted into a 30-ml stoppered centrifuge tube, made basic with 1–2 drops of 1 *N* sodium hydroxide, and extracted with two portions of ether (10 and 5 ml) with shaking for 5 min. After decantation or centrifugation, as necessary, the ether solution was removed by aspiration and filtered on anhydrous sodium sulfate into a conical bottom 20-ml tube.

The ether was evaporated in a current of nitrogen, and the residue was dissolved in 0.2 ml of carbon disulfide. About 2 μl of the solution was injected into the gas chromatograph. This operation was repeated at least three times. The correction factor was calculated as follows:

$$f = \frac{H_d C_h}{H_h C_d} \qquad \text{(Eq. 1)}$$

where f = correction factor, H_d = height of the diphenylpyraline peak, H_h = height of the hexadiphane peak, C_d = diphenylpyraline hydrochloride concentration, and C_h = hexadiphane hydrochloride concentration.

The linearity of the response was checked by keeping constant the concentration of the internal standard (0.16 mg/ml) and varying the hexadiphane concentration (0.05–0.6 mg/ml). The calibration curve was made by plotting the hexadiphane/diphenylpyraline peak height ratio against the amount of hexadiphane.

Procedure—For the liquid preparation, virtually the same procedure as described for the determination of f was followed. A solution equivalent to 400 μg of hexadiphane hydrochloride and 2 ml of the internal standard solution were pipetted into a 30-ml stoppered centrifuge tube and extracted with two portions of ether (10 and 5 ml). The ether was then discarded. This extraction was

Table II—Assay Results of the Hexadiphane–Oxazepam Hemisuccinate Combination (Hexadiphane Maleate, 3.2 mg/Capsule)

Analysis Number	Titrimetric Method		GLC Method	
	Found, mg	Recovered, %	Found, mg	Recovered, %
1	3.26	101.9	3.24	101.2
2	3.22	100.6	3.12	97.5
3	3.28	102.5	3.20	100.0
4	3.22	100.6	3.16	98.7
5	3.25	101.5	3.26	101.9
6	3.24	101.2	3.19	99.7
Mean	3.245		3.195	
SD	0.0235		0.051	
RSD, %	0.724		1.596	
$F = 4.78$	$t = 2.18$			

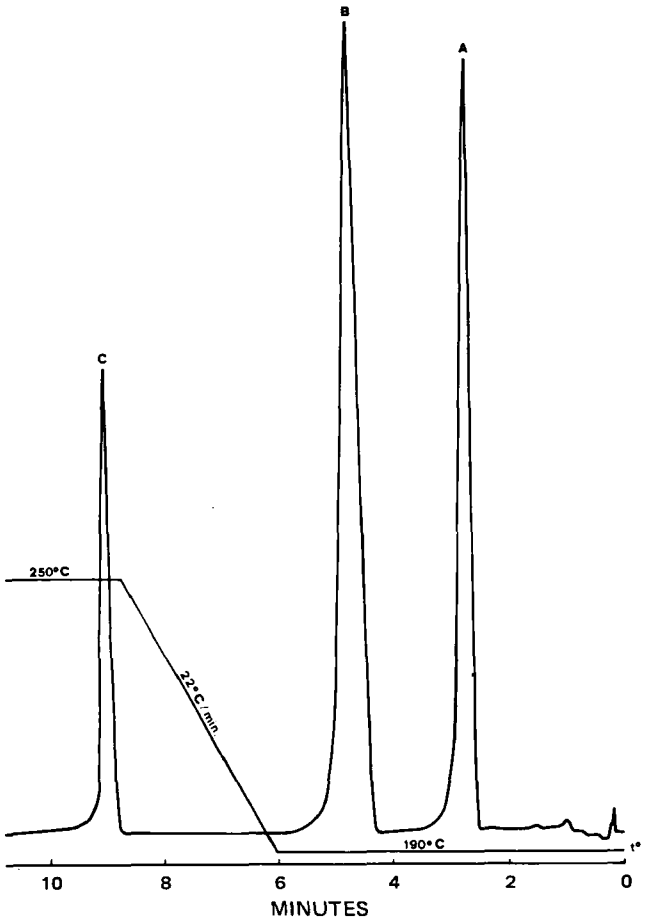


Figure 1—*Typical chromatogram. Key: A, diphenylpyraline; B, hexadiphane; and C, 1,1-diphenyl-3-hexamethyleneiminobutyronitrile.*

adopted to remove ether-soluble substances which might interfere in the analysis. After alkalinization with 1 *N* sodium hydroxide, the procedure was the same as that employed for determining *f*.

The solid products were finely pulverized. An accurately weighed quantity of powder equivalent to about 4 mg of hexadiphane hydrochloride or 6 mg of hexadiphane maleate was transferred into a 50-ml volumetric flask, and about 40 ml of 0.1 *N* hydrochloric acid was added. After heating at 70–80° and shaking for 30 min, the solution was allowed to cool. It then was brought to volume with 0.1 *N* hydrochloric acid, thoroughly mixed, and centrifuged. Five milliliters of this solution and 2 ml of the internal standard solution were pipetted into a 30-ml stoppered centrifuge tube, and the procedure described for the liquid preparation was then followed. The amount of hexadiphane hydrochloride in the sample analyzed was determined by:

$$\text{hexadiphane hydrochloride} = \frac{H_h C_d}{H_d} f \qquad \text{(Eq. 2)}$$

The value was multiplied by 1.241 to obtain the corresponding hexadiphane maleate content.

Table III—Assay Results of the Hexadiphane–Digestive Enzymes–Dimethylpolysiloxane Combination (Hexadiphane Hydrochloride, 2 mg/Tablet)

	GLC Method	
Analysis Number	Found, mg	Recovered, %
1	2.03	101.5
2	1.94	97.0
3	1.97	98.5
4	1.98	99.0
5	2.01	100.5
6	2.02	101.0
Mean	1.991	
SD	0.0375	
RSD, %	1.883	

RESULTS AND DISCUSSION

The liquid preparation and the combination with oxazepam hemisuccinate were assayed by titration with sodium lauryl sulfate and GLC. Since there was no interference from other basic nitrogen compounds, the titration was possible without additional separations.

Direct titration could not be done on the combination with digestive enzymes because of the complexity of the formulation. Only GLC analysis was performed.

The GLC results (Tables I–III) show that the method is sufficiently accurate and precise for use in the analysis of pharmaceutical preparations. Although the standard deviation of the GLC results is higher than that of the titrimetric results, the *t* test for the means and the *F* test for the variances (Tables I and II) indicate no significant difference at the 5% level.

GLC analysis, however, is preferable because of its simplicity, rapidity, and selectivity; as shown in Fig. 1, there is sharp separation of hexadiphane from its precursor II. In the absence of additional separations, the presence of this precursor would not have been revealed by titration and other chemical methods.

These features make the GLC method suitable for routine checks and those carried out during synthesis and manufacture as well as for purity checks.

Carbon disulfide was chosen as the solvent because of its relatively low response with the flame-ionization detector.

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