Table II—Intraassay Precision of Hydralazine Assay

Hydralazine Added ng/ml	Peak Height Ratio[a]	Hydralazine Found, ng/ml[b]
1	0.120 ± 0.022	1.14
2	0.210 ± 0.027	1.91
8	0.827 ± 0.031	7.22
20	2.24 ± 0.186	19.4
40	4.57 ± 0.245	39.4
80	9.54 ± 0.440	82.2
160	18.6 ± 0.875	160.

[a] Mean ± *SD*, $n = 4$. [b] Based on regression analysis of peak height ratio *versus* added hydralazine concentration weighted by the reciprocal of the peak height ratio.

zine when assayed in this manner. Thus, a plasma concentration of 12.5 μM (2.9 μg/ml) of this hydrazone would be required to yield 1 ng/ml of hydralazine. After multiple 1-mg/kg doses of oral hydralazine, peak hydralazine pyruvic acid hydrazone concentrations averaged only ~2.5 μM, range: 0.9–6.0 μM (9).

Steady-state predose apparent hydralazine concentrations up to 17 μM have been reported for patients with low creatinine clearances who received ≤200 mg of hydralazine hydrochloride daily (11), using a nonselective GLC assay (12) which converts both hydralazine pyruvic acid hydrazone and hydralazine to tetrazolophthalazine (4, 5). However, even this high concentration of acid-labile hydrazones, if composed primarily of hydralazine pyruvic acid hydrazone, would yield less than 2 ng/ml of hydralazine when the current assay is used. Other hydralazine adducts, such as the acetone, α-ketoglutaric acid, and acetaldehyde hydrazones, are less stable, but have not been detected in significant concentrations in plasma (2) or whole blood[9].

Approximately 10% of the α-ketoglutaric acid hydrazone adduct has been reported to be converted to apparent hydralazine in a selective assay procedure (2), while the pyruvic acid and acetone hydrazones did not yield apparent hydralazine. The lowest detectable hydralazine concentration and the stability of the acetaldehyde hydrazone using this procedure were not reported.

The assay procedure described here, as well as other recently published methods applicable to plasma (1–3), are much more selective than older procedures. However, the lability of the various known and potential circulating metabolites of hydralazine makes it difficult to believe that any procedure will be completely selective for unchanged hydralazine. The procedure for measuring hydralazine in whole blood is being evaluated in hypertensive patients with renal failure and in patients with congestive heart failure since these individuals are likely to have the highest concentrations of circulating metabolites.

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Quantitation of Meperidine Hydrochloride in Pharmaceutical Dosage Forms by High-Performance Liquid Chromatography

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Abstract □ A high-performance liquid chromatographic (HPLC) method for the quantitative determination of meperidine hydrochloride in pharmaceutical dosage forms was developed. The method is reproducible and precise with relative standard deviations (based on six readings) of 1.2% with hydroxyzine and 0.93% with hydroxyprogesterone caproate as the internal standards. A variety of other active and inactive ingredients which were mixed with meperidine hydrochloride did not interfere with the assay procedure. Among the ingredients tested were acetaminophen, atropine sulfate, disodium edetate, metacresol, phenol, promethazine, and sodium metabisulfite. This method appears to be stability-indicating since a hydrolyzed sample of meperidine showed zero potency and a new peak with a different retention time.

Keyphrases □ Meperidine hydrochloride—quantitation in pharmaceutical dosage forms by high-performance liquid chromatography □ Pharmaceutical dosage forms—quantitation of meperidine hydrochloride by high-performance liquid chromatography □ High-performance liquid chromatography—quantitation of meperidine hydrochloride in pharmaceutical dosage forms

Meperidine hydrochloride (ethyl 1-methyl-4-phenylisonipecotate hydrochloride) is widely used as a narcotic analgesic. In addition to single ingredient dosage forms, meperidine is also mixed with acetaminophen, atropine sulfate, and promethazine hydrochloride in commercial dosage forms.

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Table I—Assay Results of Various Commercial Dosage Forms and Synthetic Mixtures

Dosage Form	Conc. of Meperidine HCl, mg/ml or per Tablet	Other Ingredients, mg/ml	Percent[a] of the Label Claim	
			Hydroxyzine	Hydroxyprogesterone Caproate
Ampule	25.0	—	99.2	100.1
Ampule	50.0	—	99.9	100.1
Ampule	75.0	—	98.4	99.6
Ampule	100.0	—	100.3	99.2
Syrup	10.0	[b]	98.8	99.1
Tablet	50.0	[b]	99.5	100.0
Vial	50.0	Metacresol 1	99.1	98.7
Synthetic Mixture 1	50.0	Promethazine hydrochloride 50	100.8[c]	100.3
Synthetic Mixture 2	50.0	Atropine sulfate 0.4	100.7	100.6
Synthetic Mixture 3	50.0	Phenol 20, Disodium edetate 3, $CaCl_2$ 1 and sodium metabisulfite 5	99.1	99.4
Synthetic Mixture 4	50.0	Metacresol 1	99.2	99.0
Synthetic Mixture 5	50.0	Acetaminophen 300	99.8	99.2

[a] The percent relative standard deviations based on six readings were 1.2 and 0.93 with hydroxyzine and hydroxyprogesterone caproate as the internal standards, respectively. [b] The excipients were not disclosed on the label. [c] The concentration of promethazine hydrochloride was determined to be 99.1% of the label claim. It was determined by comparing the peak heights of assay and the standard mixtures.

The USP-NF method (1) for the quantitation of meperidine hydrochloride is based on the nonaqueous titration with perchloric acid. Promethazine which is mixed with meperidine in a commercial dosage form[1] is also assayed (2) using the nonaqueous titration with perchloric acid. The method is nonspecific and cannot make a distinction between meperidine and promethazine or other weak bases.

A GLC (3) method for the quantitation of meperidine in plasma has been reported with a relative standard deviation of 9.9%. A suitable high-performance liquid chromatographic (HPLC) method for the analysis of meperidine has not been described.

The purpose of these investigations was to develop an HPLC method for the quantitation of meperidine hydrochloride in pharmaceutical dosage forms which would be useful in the presence of other ingredients.

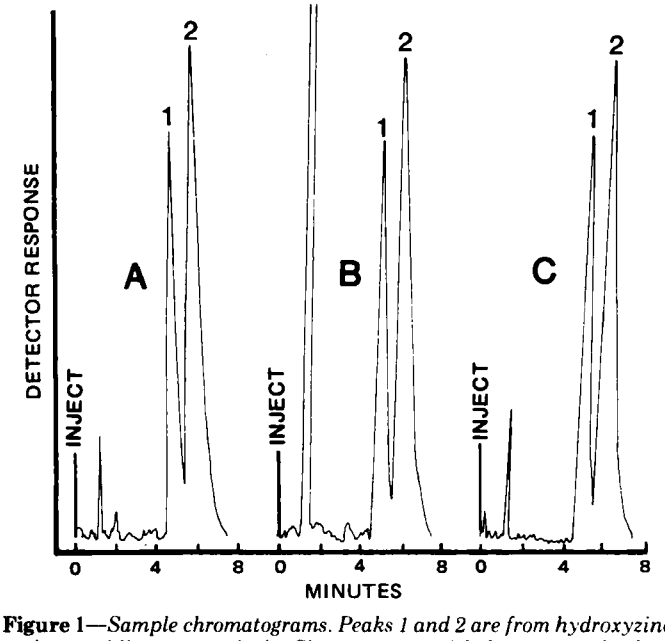

Figure 1—*Sample chromatograms. Peaks 1 and 2 are from hydroxyzine and meperidine, respectively. Chromatogram A is from a standard solution; B from a hydrolyzed sample of meperidine (see* Experimental*) and C from an ampule (number 3 in Table I). For chromatographic conditions, see text.*

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were either USP, NF, or ACS quality and used without further purification. Hydroxyprogesterone caproate[2], hydroxyzine hydrochloride[3], meperidine hydrochloride[4], and promethazine hydrochloride[5] were used as received.

An HPLC[6] equipped with a multiple wavelength detector[7], a recorder[8], and an integrator[9], was used. A μ-Bondapak-C_{18} column[10] (30 cm × 4 mm i.d.) was used at ambient temperature.

Chromatographic Conditions—The mobile phase contained 60% by volume of acetonitrile in 0.02 *M* aqueous solution of ammonium acetate. The flow rate was 2.0 ml/min (3.0 ml/min when hydroxyprogesterone caproate was the internal standard), the sensitivity was 0.04 AUFS at 232 nm, and the chart speed was 30.5 cm/hr.

Preparation of Stock and Standard Solutions—The stock solutions of hydroxyzine hydrochloride, meperidine hydrochloride, and promethazine hydrochloride were prepared by dissolving 100.0 mg of the powder in enough water to make 100.0 ml. The stock solutions were diluted further with water as needed. The standard solution contained 500.0 μg/ml of meperidine hydrochloride and 28 μg/ml of hydroxyzine hydrochloride. A standard mixture containing 250.0 μg/ml each of meperidine hydrochloride and promethazine hydrochloride and 14.0 μg/ml of hydroxyzine hydrochloride was also prepared.

The solutions of acetaminophen (~3 mg/ml), atropine sulfate (20 μg/ml), calcium chloride (10 μg/ml), disodium edetate (30 μg/ml), metacresol (10 μg/ml), phenol (200 μg/ml), and sodium metabisulfite (50 μg/ml) in water were also prepared to determine the possible interference from these ingredients, since they are added to commercial dosage forms.

Preparation of Assay Solutions, Injections (Ampules or Vials)—To an appropriate quantity of the injection containing 50.0 mg of meperidine hydrochloride, 2.8 ml of hydroxyzine hydrochloride stock solution was added and the mixture was diluted to 100.0 ml with water.

Assay solutions were made from syrup (10 mg/ml) by mixing 2.5 ml of the mixture with 1.4 ml of the stock solution of hydroxyzine hydrochloride and diluting to 50.0 ml with water, and from 50-mg tablets by weighing 20 tablets accurately and grinding them to a fine powder. An appropriate quantity of the powder containing 50.0 mg of meperidine hydrochloride was mixed with 50 ml of water and 2.8 ml of the stock solution of hydroxyzine hydrochloride. The mixture was brought to a volume (100.0

[1] Mepergan by Wyeth Laboratories, Philadelphia, Pa.
[2] E. R. Squibb Research Institute, Princeton, N.J.
[3] Pfizer Laboratories, New York, N.Y.
[4] Winthrop Laboratories, New York, N.Y.
[5] Wyeth Laboratories, Philadelphia, Pa.
[6] ALC 202 equipped with U6K universal injector, Waters Associates, Milford, Mass.
[7] Spectroflow monitor SF770, Schoeffel Instruments, Westwood, N.J.
[8] Omniscribe 5213-12, Houston Instruments, Austin, Tex.
[9] Autolab Minigrator, Spectra-Physics, Santa Clara, Calif.
[10] Waters Associates, Milford, Mass.

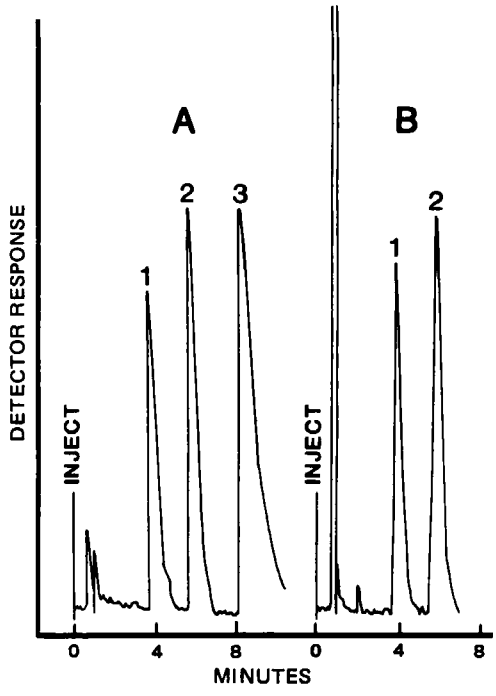

Figure 2—*Sample chromatograms. Peaks 1–3 are from meperidine, hydroxyprogesterone caproate, and promethazine, respectively. Chromatogram A is from a standard mixture containing (in μg per ml): meperidine hydrochloride (310), hydroxyprogesterone caproate (30), and promethazine hydrochloride (50). Chromatogram B is from a syrup with 350 μg/ml of meperidine hydrochloride and 30 μg/ml of hydroxyprogesterone caproate. For chromatographic conditions, see text. Flow rate was 3.0 ml/min.*

ml) with water and then filtered. The first 20 ml of the filtrate was discarded and the remainder collected for the assay.

Modifications with Hydroxyprogesterone Caproate as the Internal Standard—A stock solution of hydroxyprogesterone caproate was prepared by dissolving 100.0 mg of the powder in enough ethanol to make 100.0 ml. The standard solutions were prepared by mixing 3.0 ml of the stock solution with 60 ml of acetonitrile, an appropriate quantity of the meperidine hydrochloride stock solution concentrated (2.0 mg/ml), and enough water to make 100.0 ml. A similar procedure was used to prepare the assay solutions. In the case of tablets, only 30 ml of water was used instead of 50 ml in the extraction procedure. The assay solutions were filtered if necessary.

Assay Procedure—A 20 μl aliquot of the sample was injected into the chromatograph using the described conditions. For purpose of comparison, an identical volume of the standard solution–mixture was injected after the assay material was eluted.

Because preliminary investigations indicated that the ratio of peak heights (meperidine–hydroxyzine or meperidine–hydroxyprogesterone caproate) were directly related to the concentrations (5–13-μg range tested), the results were calculated using a standard curve or the following equation:

$$\frac{(R_{ph})_a}{(R_{ph})_s} \times 100 = \text{percent of the label claim}$$

where $(R_{ph})_a$ is the ratio of the peak heights of the assay solution and $(R_{ph})_s$ is that of the standard solution. The results are presented in Table I and Figs. 1 and 2.

In other experiments a 6.25-ml solution of meperidine hydrochloride (2 mg/ml) was mixed with 1 ml of ~1 *N* NaOH in a 25-ml volumetric flask. The flask (with lid on) was stored in an electric oven at 50° (±1°) for 48 hr. After cooling the mixture, 1 ml of ~1 *N* HCl and 0.7 ml of the stock solution of hydroxyzine hydrochloride were added. The mixture was brought to volume with water and assayed using the procedure described above. The results are presented in Fig. 1B.

RESULTS AND DISCUSSION

The results indicate (Table I, Figs. 1 and 2) that this HPLC method can be used for the quantitative determination of meperidine hydrochloride in pharmaceutical dosage forms. The results on different days were highly reproducible and precise. The relative standard deviations based on six readings were 1.2% with meperidine hydrochloride and 0.93% with hydroxyprogesterone caproate, as the internal standard, respectively. The ratio of the peak heights was directly related to the concentrations of meperidine hydrochloride (5–13-μg range tested).

A variety of other active and inactive ingredients, which are usually mixed with meperidine in commercial dosage forms, did not interfere with the assay procedure. The concentrations (in μg) tested (with 10 μg of meperidine hydrochloride) were: acetaminophen, 60; atropine sulfate, 0.4; calcium chloride, 0.2; disodium edetate, 0.6; metacresol, 0.2; phenol, 4; promethazine hydrochloride, 10; and sodium metabisulfite, 1. In addition to these ingredients, the excipients in syrup such as flavors and preservative(s) eluted immediately after the solvent peak (Fig. 2B) and did not interfere with the assay procedure. The exact nature of the excipients added was not disclosed on the label.

Since meperidine did not separate completely from hydroxyzine (Fig. 1), another internal standard (hydroxyprogesterone caproate) was developed later in these investigations. The results obtained with this internal standard were similar (Table I) to those with hydroxyzine as the internal standard. Hydroxyprogesterone caproate separated completely from meperidine and promethazine (Fig. 2) and is recommended as the internal standard. The relative standard deviations based on six readings were 0.93 *versus* 1.2% with hydroxyzine hydrochloride (an alternative internal standard). To keep hydroxyprogesterone caproate in solution, it was necessary to add 60% (v/v) of acetonitrile (same concentration as in the mobile phase) to the standard/assay solutions. The flow rate was increased to 3.0 ml/min to hasten the assay procedure.

It was possible to determine the concentration of promethazine hydrochloride (Table I, footnote *c* and Fig. 2A) present in the synthetic mixture similar to a commercial dosage form[1]. The promethazine eluted ~4.5 min later (peak 3 in Fig. 2A) than meperidine (peak 1 in Fig. 2A).

The developed method appears to be stability-indicating, since a sample of meperidine which was hydrolyzed to the free acid (see *Experimental*) showed almost zero potency (Fig. 1B), and the product(s) of decomposition eluted immediately after the solvent. The *N*-oxide, another possible degradation product, was not chromatographed.

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