Table II—Hypoglycemic Effects of 1 U of Subcutaneously Administered Regular Insulin and Sodium Insulin

Assay No.	Nadirs of Blood Glucose Levels, mg/dl		Time of Nadir, min	
	Regular Insulin	Sodium Insulin	Regular Insulin	Sodium Insulin
(1)	32	47	100	148
(2)	46	40	90	190
(3)	27	43	130	195
(4)	24	42	130	195
(5)	50	48	100	145
(6)	45	32	80	105
(7)	38	41	80	162
Mean	37.4	41.8	101.4	162.9
±1 *SD*	10.1	5.3	21.2	33.4
p	*NS*		<0.001	

subcutaneously. The site of injection was always 2.54 cm from the center of the third nipple from the head. The glucose monitor measured the lowering of the blood glucose level. The nadir blood glucose level and the time of its occurrence were noted.

This procedure was repeated in each dog, after a rest period of 1 or 2 weeks, using 1 U of regular insulin instead of 1 U of sodium insulin.

RESULTS AND DISCUSSION

The results of the six repeated bioassays of a solution containing 0.6 U of sodium insulin as measured by immunoassay are shown in Table I. The mean biological activity of sodium insulin was equal to its immunoactivity. The coefficient of variation of the six consecutive immunoassays of sodium insulin was 3%.

The hypoglycemic effects of subcutaneously administered sodium insulin and regular insulin are compared in Table II. There was no significant difference between the nadirs of blood glucose following the two forms of insulin, but the nadir induced by sodium insulin occurred significantly later than the nadir induced by regular insulin.

Chemical manipulation of a biologically active molecule can have a significant effect on its biological as well as its immunological activity. The two effects may differ, leading to a new relationship between the two activities.

We have found that sodium insulin, a new dosage form of insulin, has the same ratio of biological *versus* immunological activity as the USP reference standard insulin.

Even though the biological effect of intravenously injected sodium insulin was equal to the effect of regular insulin, there was a significant difference between the effects of the two dosage forms of insulin when administered subcutaneously. The difference between the activity of subcutaneously administered sodium insulin and regular insulin, therefore, should be attributed only to the difference in the rate at which they are absorbed into the bloodstream.

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Determination of Hydralazine in Human Whole Blood

T. M. LUDDEN *‡x, L. K. LUDDEN ‡, K. E. WADE ‡, and S. R. B. ALLERHEILIGEN *‡

Received January 18, 1982, from the **College of Pharmacy, University of Texas-Austin* and the ‡*Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.* Accepted for publication June 2, 1982.

Abstract □ The time required for the separation of plasma from the cellular components of blood can permit the *in vitro* loss of hydralazine. Thus, a high-performance liquid chromatographic (HPLC) procedure for the measurement of hydralazine in blood has been developed. 4-Methylhydralazine was used as an internal standard. The addition of *p*-anisaldehyde led to the formation of the *p*-anisaldehyde hydrazones of hydralazine and the internal standard. HPLC on a reverse-phase cyano column provided an analytical procedure in which the average relative standard deviation over the concentration range of 1–160 ng/ml was 8.3%. Hydralazine pyruvic acid hydrazone, a known circulating metabolite of hydralazine, yielded only 0.05 mole % hydralazine when submitted to this assay procedure.

Keyphrases □ Hydralazine—whole blood, derivatization, *p*-anisaldehyde hydrazones of hydralazine, analysis, high-performance liquid chromatography □ Whole blood—analysis, hydralazine, high-performance liquid chromatography □ High-performance liquid chromatography—hydralazine, whole blood analysis, *p*-anisaldehyde hydrazone of hydralazine

Selective assays for the measurement of hydralazine in plasma have been recently described (1–3). A major disadvantage of these procedures is the time required for separation of plasma from the cellular components of blood. Hydralazine disappears rapidly from plasma or blood *in vitro* (2, 4–6). Rapid sample processing at reduced temperature has been used to slow the loss prior to derivatization (7–9). The measurement of hydralazine in whole blood would circumvent this problem. In addition, because the clearance of hydralazine is high (7, 8), blood hydralazine concentrations are extremely useful for pharmacokinetic studies. However, attempts to measure hydralazine in whole blood using previously published techniques for plasma (1, 3) resulted in fouling of the chromatographic column after only a few sample injections. This paper describes a high-performance liquid chromatographic (HPLC) assay for the determination of hydralazine in whole blood using a different chromatographic column and a less polar extraction solvent.

EXPERIMENTAL

Reagents and Chemicals—Hexane, methanol, and acetonitrile were purchased as glass-distilled solvents. The acetonitrile was filtered before

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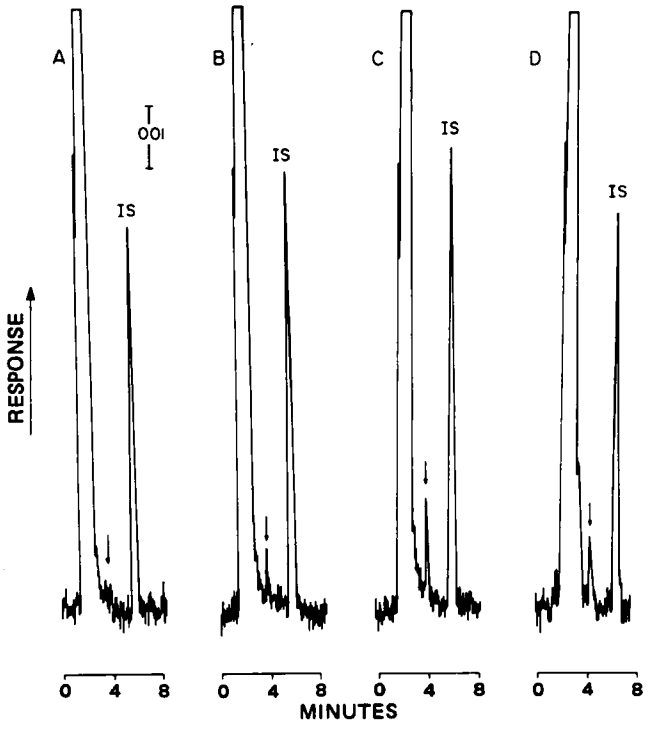

Figure 1—*Chromatograms of derivatized 3-ml blood samples containing 40 ng/ml of internal standard and (A) 0, (B) 1, (C) 2 ng/ml of hydralazine; (D) a sample obtained 150 min after administration of a 1-mg/kg oral dose of hydralazine hydrochloride to a hypertensive patient.*

being used. Hexane, hydralazine hydrochloride[1], 4-methylhydralazine[2], 4-(2-acetylhydrazino)phthalazin-1-one[3], and *p*-anisaldehyde[4] were used as received. Reference samples (3-methyl-*s*-triazolophthalazine, 4-hydrazinophthalazin-1-one) and hydralazine hydrazones of pyruvic acid, α-ketoglutaric acid, acetaldehyde, acetone, and *p*-anisaldehyde were prepared, and their identity and purity verified by gas chromatography–mass spectrometry as previously described (1, 10).

Standard Solutions—No pretreatment of the glassware was used. Hydralazine hydrochloride (61.4 mg) equivalent to 50 mg of the free base, was diluted to 50 ml with normal saline to give a concentration of 1 mg/ml. This stock solution was prepared daily. Standard A was prepared by diluting 0.6 ml of the stock solution to 10 ml with normal saline to yield a concentration of 60 ng/μl. Standard B was prepared by diluting 0.5 ml of Standard A to 10 ml with normal saline resulting in a concentration of 3 ng/μl. The internal standard solution was prepared by dissolving 50 μg of 4-methylhydralazine in 10 ml of 0.01 *N* HCl.

Standard Curve Samples—Three milliliters of whole blood containing EDTA (ethylenediaminetetraacetic acid) as an anticoagulant was placed in a 35-ml centrifuge tube fitted with a polytef-lined screw cap. After addition of 20 μl of *p*-anisaldehyde, appropriate volumes of either standard A or B were added along with 8 μl (40 ng) of the internal standard solution. The tube was capped immediately and mixed for 15 sec on a vortex mixer. Each sample was prepared individually to minimize the intervals between additions. Samples were allowed to stand at room temperature for 10 min. Preliminary studies indicated that 10 min is optimal for the formation of the *p*-anisaldehyde hydrazones of hydralazine and the internal standard. After derivatization, 10 ml of hexane was added and the sample was placed immediately on a reciprocal shaker[5] (180 strokes/min) for 10 min.

Phases were separated by centrifugation at 1000×*g* for 10 min. The organic phase was transferred to a 15-ml conical centrifuge tube and the solvent removed under a nitrogen stream. The remaining aqueous phase was processed as previously described (3) in order to measure the hydralazine pyruvic acid hydrazone concentration.

Chromatography—Each extraction residue was diluted with 100 μl of methanol and the entire volume was injected onto a reverse-phase column (3.9-mm i.d. × 30 cm)[6]. The mobile phase was 70% (v/v) acetonitrile in 0.15 *M* sodium acetate buffer, pH 3.0, pumped[7] at a flow rate of 2 ml/min. Detection[8] was at 365 nm.

Table I—Conversion of Hydralazine Hydrazones to Derivatized Hydralazine During the Analytical Process

Hydrazone	Initial Hydrazone Concentration, μg/ml	Hydralazine *p*-Anisaldehyde Hydrazone Found, mole %[a]
Pyruvic Acid	5	0.05
α-Ketoglutaric Acid	1	0.15
Acetone	1	4.2
Acetaldehyde	1	27.1

[a] Mean of two determinations.

Assay Evaluation—The efficiencies of derivatization and extraction were determined as previously described for plasma (1, 3) using fresh, whole human blood. Selectivity was evaluated by adding known or potential metabolites to whole blood and immediately assaying for hydralazine. Intraassay precision was determined by assaying multiple standard samples containing hydralazine concentrations of 1, 2, 8, 20, 40, 80, and 160 ng/ml.

Patient Samples—Blood, 3 ml, was drawn rapidly and placed in a 35-ml centrifuge tube containing EDTA. The internal standard and 20 μl of *p*-anisaldehyde were added immediately. The time between blood drawing and the addition of derivatizing reagent was <30 sec. After derivatization, samples were immediately extracted and prepared for chromatography as described for standard curve samples.

RESULTS

The retention times for hydralazine *p*-anisaldehyde hydrazone and 4-methylhydralazine *p*-anisaldehyde hydrazone were 3.5 and 6 min, respectively. Typical chromatograms are shown in Fig. 1. Maximum sensitivity for purposes of quantitation was ~1 ng/ml using a 3-ml blood sample. Derivatization efficiency for hydralazine was 103 ± 9% (mean ± *SD*) and extraction recovery was 70.5 ± 1.9%. The 3-methyl-*s*-triazolophthalazine, 4-hydrazinophthalazin-1-one, and 4-(2-acetylhydrazino)phthalazin-1-one yielded no chromatographic peaks when assayed with this procedure.

The acetaldehyde and acetone hydrazones yielded peaks at 4.1 and 2.9 min, respectively, which correspond to the retention times for standards of these materials. These hydrazones, as well as the hydrazones of α-ketoglutaric acid and pyruvic acid, yielded various quantities of derivatized hydralazine (Table I). The pyruvic acid hydrazone, the only one of the four hydrazones known to be present in the circulation (2–4), yields the least amount of apparent hydralazine. The acetaldehyde hydrazone is particularly labile. No significant quantities of this hydrazone or the hydrazones of α-ketoglutaric acid or acetone have been found in humans (2). These four hydrazones have similar stabilities in plasma (Table I).

The results of the intra-assay precision study are shown in Table II. The mean relative standard deviation was 8.3%. Precision is somewhat decreased at the lower concentrations, particularly at 1 ng/ml. This assay has been applied to blood from several patients receiving various drugs, including propranolol, furosemide, hydrochlorthiazide, digoxin, and nitroglycerin, without evidence of interference.

DISCUSSION

The assay described here has two advantages over other recently published techniques for the determination of hydralazine (1–3). First, it can be used to measure hydralazine concentrations in whole blood. This eliminates the time required to separate plasma, during which a significant fraction of hydralazine can continue to react with pyruvic acid to form the corresponding hydrazone. The use of whole blood also decreases the total blood volume required per determination. Second, the sensitivity of the assay has been extended to 1 ng/ml of blood when a 3-ml blood sample is used.

Hydralazine pyruvic acid hydrazone yields only 0.05 mole % hydrala-

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Ciba-Geigy Co., Summit, N.J.
[3] Ciba-Geigy, Ltd., Basel, Switzerland.
[4] Eastman Kodak Co., Rochester, N.Y.
[5] Eberbach Horizontal Shaker.
[6] μBondapak CN, Waters Associates, Milford, Mass.
[7] Model 6000, Waters Associates, Milford, Mass.
[8] Model 970, Tracor, Austin, Tex.
[9] Unpublished results.

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

V. DAS GUPTA

Received January 22, 1982, from the *College of Pharmacy, University of Houston, Houston, TX 77030.* Accepted for publication June 2, 1982.

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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