Table I—Predicted and Observed First-Pass Metabolism of Nortriptyline in Six Subjects

Subject	AUC, μg/liter × hr	% FP (Observed)	% FP (Predicted) Blood Flow Model	% FP (Predicted) Plasma Flow Model
5	895	54	38	53
6	860	50	39	54
7	925	41	37	53
8	670	53	45	61
9	885	49	38	54
10	1500	50	27	41
Mean ± SEM	—	49.50 ± 1.88	37.33 ± 2.38	52.67 ± 2.64
T, predicted:observed		—	4.02 $p<0.0025$	0.98 (n.s.)

ipramine using a blood flow model, indicating that the plasma indeed represents the total blood concentration. A similar approach is made here using both blood and plasma flow models for nortriptyline.

EXPERIMENTAL

The extent of the first-pass effect was calculated, using Eq. 1, from the *AUC* values reported following oral administration of 50 mg of nortriptyline to six subjects (1). A mean blood flow of 91.8 liters/hr (4) and a plasma flow of 48.65 liters/hr were used for these calculations (hematocrit = 0.47).

RESULTS

Table I lists the predicted and experimentally observed values of the first-pass metabolism of nortriptyline in humans. The blood flow model, with the assumption that the plasma concentration represents the whole blood concentration, fails to predict the first-pass metabolism, whereas the plasma flow model accurately describes the first-pass metabolism. If it is assumed that the absorption of nortriptyline is complete and that there is no extrahepatic metabolism of nortriptyline, it can be concluded that the clearance takes place mainly from the plasma, which does not represent the whole blood concentration.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 29, 1975, from the *Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*

Accepted for publication December 8, 1975.

Liquid Chromatography in Pharmaceutical Analysis V: Determination of an Isoniazid–Pyridoxine Hydrochloride Mixture

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Abstract □ Operating parameters are described for the qualitative and quantitative analysis of an isoniazid–pyridoxine hydrochloride mixture by high-pressure liquid chromatography. Each compound was chromatographed on an octadecyl column, using absolute methanol–water (60:40) (pH 2.5) containing 0.01 *M* dioctyl sodium sulfosuccinate. The flow rate was 2.0 ml/min (2500 psig), and the peaks were detected at 293 nm. The analysis was accomplished using ion-pair formation for effecting chromatographic separation. The time required for separation of the drug mixture is approximately 12 min with an accuracy of 0.17–0.30%.

Keyphrases □ High-pressure liquid chromatography—analysis, isoniazid and pyridoxine hydrochloride in mixtures □ Isoniazid—high-pressure liquid chromatographic analysis in mixtures with pyridoxine hydrochloride □ Pyridoxine hydrochloride—high-pressure liquid chromatographic analysis in mixtures with isoniazid □ Antitubercular agents—isoniazid, high-pressure liquid chromatographic analysis in mixtures with pyridoxine hydrochloride □ Vitamins—pyridoxine hydrochloride, high-pressure liquid chromatographic analysis in mixtures with isoniazid

The separation and quantification of the antitubercular mixture, isoniazid–pyridoxine hydrochloride, are reported as a continuation of investigations into the use of high-pressure liquid chromatography (HPLC) in the analysis of multicomponent dosage forms. Previous studies dealt with the separation, detection, and quantification of cough–cold, diuretic–antihypertensive, and antispasmodic mixtures (1–4) by HPLC. Some analytical problems associated with the isoniazid–pyridoxine hydrochloride mixture include a 10-fold difference in concentration of the ingredients and a much greater molar absorptivity for isoniazid than pyridoxine at 254 nm, the fixed wavelength presently used in most HPLC UV detectors.

Isoniazid and pyridoxine hydrochloride have been analyzed by various methods. Techniques used for isoniazid include colorimetry (5–9), UV spectrophotometry (6), fluorescence (10), polarography (6), and titrimetry (11). Procedures for pyridoxine hydrochloride involve nonaqueous titrimetry (12), UV spectrophotometry (12), colorimetry (13–15), and fluorescence (16).

The determination of the isoniazid–pyridoxine hydrochloride mixture by HPLC overcomes or circumvents many shortcomings in the reported methods. This paper describes an analysis of the drugs using ion-pair

Table I—Calibration Data for Standard Drug Solutions

Compound	Final Concentration, mg[a]	D/IS Ratio[b]	Slope	Intercept	$r \pm s$[c]
Isoniazid	5.0	2.237 ± 0.031[d]	0.479	−0.180	0.9999
	10.0	4.580 ± 0.007			±0.0226
	20.0	9.413 ± 0.049			
Pyridoxine hydrochloride	0.5	1.386 ± 0.030	2.631	0.062	0.9999
	1.0	2.680 ± 0.010			±0.0091
	2.0	5.328 ± 0.020			

[a] Total milligrams per 10-ml sample. [b] Data represent four replicate injections of standard solutions; D/IS is the ratio of the integrated area of the drug at some concentration divided by the integrated area of isonicotinic acid at 5 mg/10 ml. [c] Correlation coefficient ± standard deviation. [d] Confidence limits at $p = 0.05$.

formation for effecting chromatographic separation. The use of ion-pairs in the separation of tartrazine and its intermediates by HPLC was reported recently (17). A quantitative study is described for a simulated dosage form. The time required for chromatographic separation of the drug mixture is approximately 12 min.

EXPERIMENTAL[1]

Reagents and Chemicals—Powdered samples of isoniazid[2] and pyridoxine hydrochloride[3] were used in the analytical procedure. In addition, dioctyl sodium sulfosuccinate[4] was a component of the mobile phase. All other chemicals and solvents were the highest commercially available materials.

Mobile Phases—The mobile phases were various concentrations of absolute methanol mixed with distilled water. Dioctyl sodium sulfosuccinate was added to make a 0.01 *M* solution, and the pH was adjusted to 2.5 with sulfuric acid. The solutions were prepared fresh daily.

Internal Standard Solution—The stock internal standard solution (50 mg/10 ml) was prepared by dissolving isonicotinic acid[5] in distilled water.

Standard Solutions for Calibration Curves—Stock solutions of isoniazid (50 mg/10 ml) and pyridoxine hydrochloride (5 mg/10 ml) were prepared in distilled water. Accurately pipetted volumes of 1.0, 2.0, and 4.0 ml of each stock solution were placed in 10-ml volumetric flasks. One milliliter of the internal standard stock solution was added to each flask, followed by the addition of distilled water to volume.

The three concentrations of each drug were subjected to a linear regression analysis, and the slope and intercept were calculated (Table I).

Conditions for Chromatographic Separation and Quantification—The degassed mobile phase was pumped through a column containing a monomolecular layer of octadecyltrichlorosilane (C_{18}), chemically bonded to a high efficiency porous silica surface[6], at a flow rate of 2.0 ml/min (2500 psig) at room temperature until a stable baseline was obtained. Replicate 40-μl injections of sample and standard solutions were made using a 100-μl syringe[7]. The chart recorder provided a record of drug elution from the column as peaks on a chromatogram. In all cases, the solute was measured by digital integration of the peak area[1].

RESULTS AND DISCUSSION

The analysis of the dosage form, isoniazid–pyridoxine hydrochloride, involved two separate analytical problems: (*a*) the development of operating parameters of HPLC that would separate the two components without peak overlap, and (*b*) the detection and quantification of each component at the level in the dosage form.

Initial attempts to separate isoniazid and pyridoxine hydrochloride were made using cation-exchange chromatography. Table II shows the effect of mobile phase pH on the separation of the two drugs as expressed in R_s values. The R_s value (18), a theoretical parameter, is a reasonable measurement of the separation of two species on a column[8]. Satisfactory quantification of a multicomponent dosage form is dependent on adequate resolution of the components. The use of cation-exchange chromatography in the separation and quantification of isoniazid–pyridoxine hydrochloride was not satisfactory because:

1. There was a marked variation in the resolution of the drugs using two batches of resin from the same supplier.
2. The sensitivity of the R_s value of the two components to changing pH, with a maximum near pH 4.5, made it difficult to develop an analytical method with reproducible resolution values on a repetitive basis.

The separation of isoniazid and pyridoxine hydrochloride on conventional reversed-phase chromatography was also unsuccessful. The affinity of the components for the stationary phase was too low. However, addition of a small quantity of dioctyl sodium sulfosuccinate to the mobile phase gave sufficient selectivity to effect the separation. Solvent composition was varied to include absolute methanol with

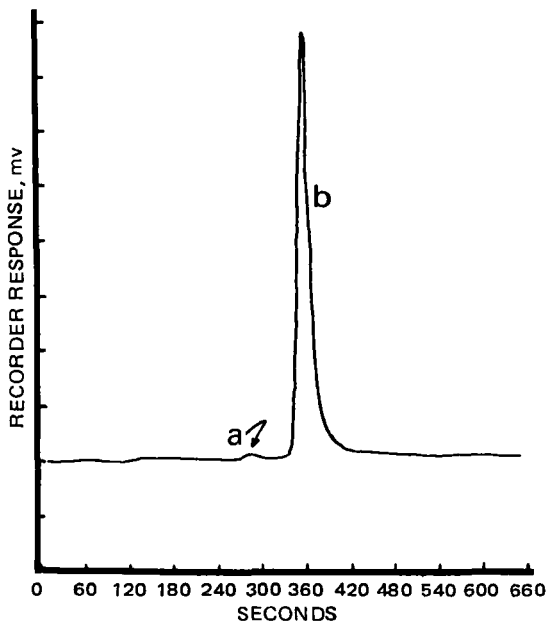

Figure 1—*Liquid chromatogram of isoniazid–pyridoxine hydrochloride (10:1) mixture at 254 nm on octadecyl column with absolute methanol–water (60:40, pH 2.5) containing 0.01* M *dioctyl sodium sulfosuccinate. Key: a, pyridoxine hydrochloride; and b, isoniazid.*

[1] A Waters Associates liquid chromatograph (model ALC/GPC 201), equipped with an M-6000 pump, a Perkin-Elmer model LC-55 variable wavelength UV–visible detector, and an Infotronics integrator (model CRS-204) with digital printout, and a Waters packed column, 4 mm i.d. × 30 cm, were used.
[2] Matheson, Coleman and Bell, East Rutherford, N.J.
[3] Nutritional Biochemicals Corp., Cleveland, Ohio.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Eastman Organic Chemicals, Rochester, N.Y.
[6] Micro-Bondapak/C_{18}, <10 μm, Waters Associates, Milford, Mass.
[7] Model B-110, Precision Sampling Corp., Baton Rouge, La.

[8] It is possible to calculate the approximate resolution (R_s) of two components by the equation $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are retention times, and w_1 and w_2 are base peak widths of Compounds 1 and 2, respectively. In past experience, two components with an R_s value >1.5 showed satisfactory resolution for quantification by this analytical technique if the peak areas were approximately equal. A significant difference in peak area for two components may require R_s values of 2 or greater.

Table II—Effect of Mobile Phase pH on Approximate Resolution (R_s) Values for Isoniazid–Pyridoxine Hydrochloride on Cation-Exchange Column

Cation-Exchange Resin[b]	pH of Mobile Phase[a]			
	3.5	4.5	5.5	6.5
Batch 1	<0.24[c]	3.08	2.15	0.36
Batch 2	0.62	4.95	2.40	1.18

[a] Mobile phase was aqueous 0.01 *M* ammonium acetate containing 0.01% sodium azide. The pH was adjusted to the desired level with acetic acid. The flow rate was 2.0 ml/min (1000 psig), and the peaks were measured using UV detection at 254 nm. [b] A sulfonated alkyl phenyl chemically bonded to solid glass cores with a porous silica surface (CX-Corasil, Waters Associates, Milford, Mass.). [c] See Footnote 8. Resolution values were measured under experimental conditions that produced equivalent peak areas for both components.

changing concentrations of distilled water. The pH of the mobile phase (pH 2.5) and of the octadecyl (lipophilic) stationary phase remained constant during the investigation.

Apparently, the separation depends on the reversible formation of ion-pairs within the chromatographic system and separation of the drugs on the basis of differences in the lipophilicity of the ion-pairs formed. The effect of changing solvent composition on retention times

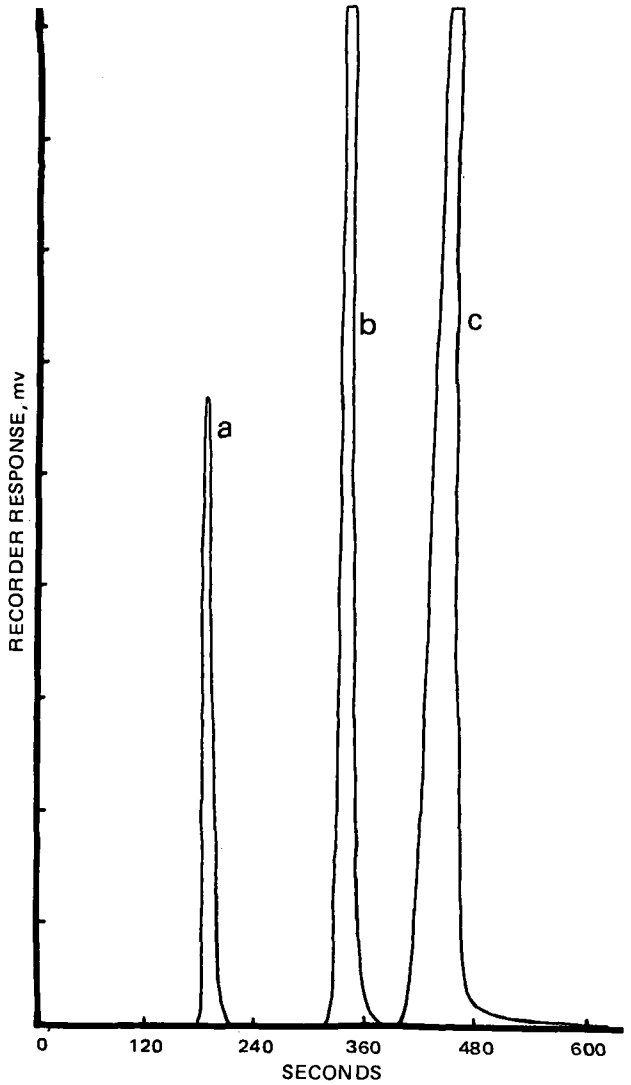

Figure 2—*Liquid chromatogram of isoniazid–pyridoxine hydrochloride mixture at 293 nm on octadecyl column with absolute methanol–water (60:40, pH 2.5) containing 0.01* M *dioctyl sodium sulfosuccinate. Key: a, isonicotinic acid; b, pyridoxine hydrochloride; and c, isoniazid.*

Table III—Effect of Mobile Phase Composition on Retention Times[a]

Compound	Mobile Phase Composition, Methanol–Water[b]			
	70:30	60:40	50:50	40:60
Isoniazid	261[a] (14)[d]	578 (60)	1332 (116)	—[c]
Pyridoxine hydrochloride	239 (37)	388 (39)	656 (60)	1228 (96)
R_s[e] =	0.86	3.84	7.68	>16.7[f]

[a] Retention time is expressed as seconds measured as elapsed time between injection and attainment of the chromatographic peak maximum. Eluted peaks were monitored using UV detection at 293 nm. [b] Solvents contained 0.01 *M* dioctyl sodium sulfosuccinate and were buffered to pH 2.5 with sulfuric acid. [c] Retention time greater than 3000 sec. [d] Base peak width expressed as seconds. [e] See Footnote 8. [f] Calculated on the basis of a retention time of 3000 sec and a base peak width of 116 sec for isoniazid.

Table IV—Analysis of Isoniazid–Pyridoxine Hydrochloride in Known Mixture

Mixture	Added, mg	Amount Found[a], mg	Accuracy, %
Isoniazid	10.0	10.017 ± 0.073[b]	0.17
Pyridoxine hydrochloride	1.0	0.997 ± 0.004	0.30

[a] Based on four replicate determinations of known mixture. [b] Confidence limits at $p = 0.05$.

and base peak widths is shown in Table III. The table also shows calculated R_s values for both drugs in various solvent systems on the octadecyl column. A mobile phase containing methanol–water (60:40) was chosen for the quantification since adequate resolution could be achieved in the shortest time.

Initially, the separation was monitored at 254 nm. However, at this wavelength, the molar absorptivity of isoniazid is much greater than that of pyridoxine hydrochloride (Fig. 1). When the wavelength of the detector was changed to 293 nm (the λ_{max} of pyridoxine hydrochloride in acid media), the detector response was approximately equivalent for the different molarities of the drugs. Therefore, 293 nm was used to facilitate the simultaneous determination of the two drugs.

Figure 2 illustrates a chromatogram of the drugs being assayed. Various concentrations of standard solutions of each drug dissolved in the absolute methanol–water (60:40) mixture containing 0.01 *M* dioctyl sodium sulfosuccinate were chromatographed using the octadecyl column. Isonicotinic acid was added to each solution as the internal standard[9]. The area under the curve for each peak on the chromatograms was evaluated with a digital integrator[1]. The ratio of each peak area to the area of the internal standard was calculated for each chromatogram. A linear regression line of these data at three concentrations of each drug gave the slope, intercept, and correlation coefficient for each calibration curve (Table I).

A known mixture containing a quantity of each drug was chromatographed, and the ratios of drug peak areas to internal standard peak areas (D/IS) were calculated for each drug. The constants (slope and intercept) for the linear regression equation (Table I) were used to solve for drug concentration [D/IS = (slope × concentration) + intercept]. The calculations were performed on a programmable calculator[10].

The data in Table IV demonstrate the quantitative results obtained for a simulated isoniazid–pyridoxine hydrochloride (10:1) dosage form. The method is also capable of detecting and subsequently quantifying a 20:1 mix of the two drugs (Table I). The utility of HPLC

[9] It has been suggested that, in certain circumstances, isoniazid might degrade to yield isonicotinic acid as a major contaminant. If this is suspected, an alternative choice for the internal standard is either nicotinic acid (retention time and base peak width of 159 and 21 sec, respectively) or nicotinamide (retention time and base peak width of 198 and 30 sec, respectively). The stock solution concentration given under *Experimental* for isonicotinic acid can be used for either compound.

[10] Olivetti-Underwood programma 101.

in the analysis of the isoniazid–pyridoxine hydrochloride (10:1) mixture is clearly demonstrated, with an accuracy of 0.17–0.30%.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 22, 1975, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30602*

Accepted for publication December 10, 1975

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Selectivity of 4-Methoxyphenethylamine Derivatives as Inhibitors of Monoamine Oxidase

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Abstract □ It has been established that the oxidative deamination of tyramine by monoamine oxidase is inhibited by (±)-4-methoxy-β-hydroxyphenethylamine and its *N*-methylated derivatives. This particular series of compounds does not inhibit the action of monoamine oxidase when tryptamine is used as the substrate. In contrast, 4-methoxyphenethylamine and its *N*-methylated homologs inhibit the monoamine oxidase-catalyzed deamination of both tyramine and tryptamine.

Keyphrases □ 4-Methoxyphenethylamine and *N*-methylated homologs—effect on monoamine oxidase-catalyzed deamination of tyramine and tryptamine □ Monoamine oxidase—deamination of tyramine and tryptamine, effect of 4-methoxyphenethylamine and *N*-methylated homologs □ Tyramine—monoamine oxidase-catalyzed oxidative deamination, effect of 4-methoxyphenethylamine and *N*-methylated homologs □ Tryptamine—monoamine oxidase-catalyzed oxidative deamination, effect of 4-methoxyphenethylamine and *N*-methylated homologs □ Enzymes—monoamine oxidase, deamination of tyramine and tryptamine, effect of 4-methoxyphenethylamine and *N*-methylated homologs □ Structure–activity relationships—4-methoxyphenethylamine and *N*-methylated homologs, effect on monoamine oxidase-catalyzed deamination of tyramine and tryptamine

4-Methoxy-β-hydroxyphenethylamine [1-(4-methoxyphenyl)-2-aminoethanol] has been detected in extracts of *Coryphantha cornifera* (DC.) Br. and R. var. *echinus* (Engelm.) L. Benson (1), while *Dolichothele longimamma* (DC.) Br. and R. was recently shown to contain *N*-methyl-4-methoxy-β-hydroxyphenethylamine [1-(4-methoxyphenyl)-2-(methylamino)ethanol] (2). These naturally occurring compounds, together with *N,N*-dimethyl-4-methoxy-β-hydroxyphenethylamine [1-(4-methoxyphenyl)-2-(dimethylamino)ethanol], have been found to inhibit the oxidative deamination of tyramine by monoamine oxidase (3). These data resulted from a screen used to correlate the pharmacological activity of cactus alkaloids and related compounds with the folkloric uses of various cacti.

Recently, it was noted that certain compounds inhibit the reaction of monoamine oxidase with tryptamine or serotonin but not with tyramine (4–7). The possibility of finding selective monoamine oxidase inhibitory activity prompted a reexamination of 4-methoxy-β-hydroxyphenethylamine and its *N*-methylated derivatives using tryptamine as the substrate.

N-Methyl-4-methoxyphenethylamine [1-(4-methoxyphenyl)-2-(methylamino)ethane] has been isolated from a number of cacti with potential psychoactivity (1, 8–12). This compound, as well as its *N*-methyl and *N*-demethyl derivatives, was tested for monoamine oxidase inhibitory activity using both tyramine and tryptamine as substrates. These studies revealed the significance of the β-hydroxy group in a series of phenethylamines with known monoamine oxidase inhibitory activity. In addition, the effects of the naturally occurring *N*-methyl-4-methoxyphenethylamine and its *N*-methyl homologs on monoamine oxidase were established.

EXPERIMENTAL

Synthesis—The synthesis of racemic 4-methoxy-β-hydroxyphenethylamine hydrochloride (I), *N*-methyl-4-methoxy-β-hy-