Table II—Precision of Assay for Trace Quantity of IIa in a Simulated Mixture

Measurement	IIa Found, %[a]
1	0.86
2	0.78
3	0.82
4	0.82
5	0.72
6	0.83
7	0.84
Average	0.81
RSD	5.7

[a] Relative to Ia + IIa = 100% and computed with a correction for detector response as described in the text.

The original chromatographic peak height ratio could then be adjusted accordingly. The net result was that the Ia peak height had to be divided by 1.25; *i.e.*, Ia showed an apparent enhancement relative to IIa by a factor of 1.25 under the assay conditions. Equivalent experiments with the acids were performed, and IIa was enhanced relative to IIb by a factor of 1.23. The reason for the difference in detector response between the epimers is not known.

Sample and reference preparations are equivalent, quick, and routine. Consecutive chromatographic injections may be repeated every 7 min, or the injections may be staggered between the drug and internal standard to effect even shorter intervals.

The silica gel column appears to be quite stable. After several weeks of constant use, it gave chromatograms insignificantly different from the one shown in Fig. 1. This finding was also true for Ib, indicating that a small amount of acetic acid in the mobile phase does not cause significant deterioration of column performance. Some variability was noted, however, between different columns. For best results, the use of a column with 4000 or more plates/0.305 m, as measured and reported by the manufacturer, is recommended. Alternatively, ethyl acetate may be substituted for methyl acetate in the mobile phase. Because the former solvent is less polar than the latter, it gives better resolution of the epimers, albeit with a longer analysis time.

Preliminary experiments showed that the chromatographic system described here has good potential for separating various prostaglandin pairs, each characterized by being epimeric at C-15. Interestingly, the 15-(*R*)-epimer always was eluted before the 15-(*S*)-epimer. This trend was noted previously for some naturally occurring prostaglandins and their C-15-epimers (4).

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Comparison of Observed and Predicted First-Pass Metabolism of Nortriptyline in Humans

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Abstract □ The extent of first-pass metabolism of nortriptyline, calculated by comparing the areas under the plasma concentration–time curves following intravenous and oral dosing in six individuals, varied from 41 to 54%. Theoretically predicted values ranged from 41 to 61% based on a plasma flow model, indicating that the clearance takes place mainly from the plasma, which does not represent the whole blood concentration.

Keyphrases □ Nortriptyline—first-pass metabolism predicted using plasma flow rates, compared to observed rates □ Metabolism, first pass—nortriptyline, predicted using plasma flow rates, compared to observed rates □ Pharmacokinetics—nortriptyline, first-pass metabolism predicted using plasma flow rates, compared to observed rates □ Antidepressant agents—nortriptyline, first-pass metabolism predicted, compared to observed rates

The systemic availability of nortriptyline in humans was recently reported to vary from 46 to 59% (1). This low bioavailability compared to intravenous dosing was attributed to the first-pass metabolism of nortriptyline, assuming complete absorption from the GI tract and no extrahepatic metabolism (1). It was also postulated (1) that the plasma concentration represents the whole blood concentration.

The purpose of this report is to show that the last assumption may not be correct, since a theoretical prediction of first-pass metabolism can be made if plasma flow rates are considered instead of total blood flow, indicating restriction of nortriptyline to plasma or slow partitioning between plasma and blood cells (2).

DISCUSSION

Theoretical prediction of the first-pass metabolism of imipramine based on the equation of Gibaldi *et al.* (2) was reported (3):

$$\% FP = \frac{(\text{dose}/AUC)(100)}{\text{flow rate} + (\text{dose}/AUC)} \qquad \text{(Eq. 1)}$$

where % *FP* is the percent of drug metabolized during each pass through the liver, *AUC* is the area under the plasma concentration–time curve following oral administration, and flow rate is the blood or plasma flow rate through the liver.

It was shown (3) that good correlations can be obtained for im-

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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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