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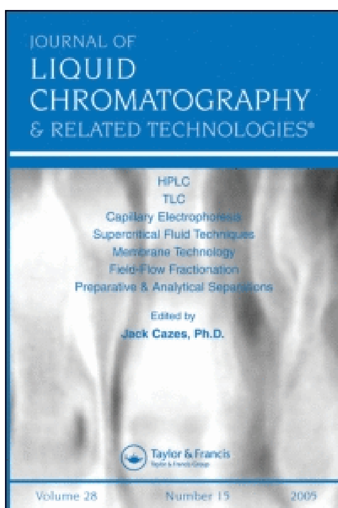

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HIGH PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF ISONIAZID AND ACETYLISONIAZID IN HUMAN PLASMA

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ABSTRACT

A quantitative high pressure liquid chromatographic (HPLC) assay has been developed for the determination of isoniazid (INH) and acetylisoniazid (ACINH) in human plasma. Plasma samples were taken from a patient after oral administration of INH (with proven tuberculosis infection). A C_{18} reversed phase radial compression column was used to separate INH and ACINH from other plasma components. The analysis takes 10 minutes per sample and the lower limit of detection for each compound is 0.10 ug/ml plasma.

INTRODUCTION

Tuberculosis (TB) is a prevalent disease in the United States with a reported incidence in 1979 of 12.6 per 100,000 population (27,669 newly diagnosed cases in one year) (1). It is estimated that there are more

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than 50,000 patients with TB at any one time. Isoniazid (INH), isonicotinic acid hydrazide. is an effective drug used in the treatment of TB. The minimal tuberculostatic concentration is 0.025 to 0.050 ug INH/ml plasma (2). An enzyme in the liver, N-acetyltransferase, is responsible for the production of the major metabolic product of ACINH (3). There is a bimodal distribution of the activity of this enzyme in the population which divides the population into rapid and slow acetylators of INH (4,5). The ACINH metabolite does not have antituberculostatic activity.

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Very few HPLC procedures have been developed for analysis of INH and ACINH. Stewart _et al_ (6) measured INH and pyrodoxime from pharmaceutical preparations and INH has also been analyzed in tablets by the method of Bailey and Abdou (7). Ion-paired chromatography was used for separation of INH and ACINH in spiked plasma and urine samples by the procedure of Saxena _et al_ (8).

In this procedure INH and ACINH were analyzed from human plasma following oral dosing of the drug. Ion-pairing was accomplished with dioctyl sulfosuccinate as the reagent. A Waters Associates C_{18} reversed phase radial compression column allowed rapid separation of drug and metabolite at high flow rates.

EXPERIMENTAL

Materials

Isoniazid was obtained from Aldrich Chemical Co. (Milwaukee, WI), and dioctyl sulfosuccinate was purchased from Sigma Chemical Co. (St. Louis, MO). Acetylisoniazid and 1-benzoyl-2-isonicotinoylhydrazine (internal standard) were synthesized by the method of Mitchell *et al* (9) and Kerr *et al* (10), respectively.

Instrumentation

A Waters Associates 202 HPLC with a 6UK injector and a radial compression column unit was used with a 11.5 cm by 0.8 cm 5 um C_{18} uBondapak column. The guard column contained 10 um C_{18} uBondapak reversed phase packing (DuPont, Wilmington, DE).

Operating Procedures and Conditions

The mobile phase consisted of 0.001 M dioctyl sulfosuccinate in distilled water/ethanol (55/45%) adjusted to pH 2.50 and degassed for 30 minutes. The column was at ambient temperature (approximately 22°C) and the flow rate set at 4.0 ml/min (2000 psi). Sample volumes of 250 ul were injected per analysis and the detector set at 254 nm.

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Sample Collection and Preparation

Informed consent was obtained from a patient who exhibited proven infection with tubercle bacillus but in otherwise good clinical status. The patient was not taking other medications at the time of the study. A heparin lock was placed in a superfical arm vein for a 12 hour sampling period. INH at a dose of 300 mg (as tablets) was given daily and 6 ml of blood withdrawn at 0, 1, 2, 4, 6, and 12 hours after dosing. Plasma was obtained by centrifugation for 10 minutes at 500 x g and 2 ml of plasma from each sampling period was diluted with 100 ul water containing 3 ug of internal standard. After addition of 2 g of ammonium sulfate and 40 ml of water saturated n-butanol-chloroform (30/70%) the samples were shaken for 10 minutes and centrifuged for 10 minutes at 500 x g. The organic layer (35 ml) was transferred into a clean tube (50 ml) and 1 ml of 0.50 N sulfuric acid was added and shaken for 10 minutes (the drug, metabolite, and internal standard were extracted into this aqueous acidic layer). The solution was then centrifuged for 10 minutes at 500 x g and 250 ul of the upper aqueous layer was injected into the HPLC.

The recovery for both compounds was 90%.

Calculations

Peak height ratios were calculated by dividing the peak height of INH or ACINH by the internal standard

peak height. Calibration curves were constructed by plotting peak height ratios (INH/internal standard or ACINH/internal standard) versus the concentration of the INH or ACINH standard (ng/ml plasma). The concentration of the drug or its metabolite was calculated from the peak height ratio using the slope and intercept obtained by a linear regression analysis of the calibration curve.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram of INH, ACINH, and internal standard isolated from human plasma 4 hours following oral dosing. The retention times for ACINH,

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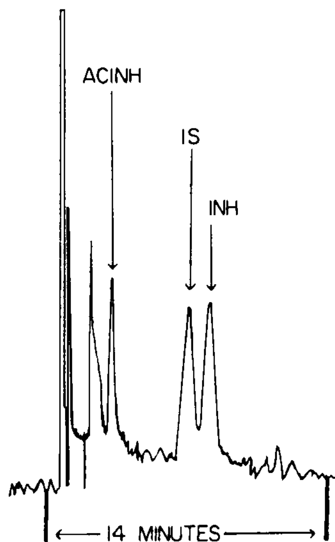

Figure 1. HPLC chromatogram of ACINH, internal standard and INH with retention times of 205, 535 and 640 seconds, respectively. Extracted from human plasma 4 hours following oral dosing with INH.

internal standard, and INH are 205, 535, and 640 seconds, respectively. Positive identification was achieved by peak superimposition, i.e., by addition of ACINH and INH standards (500 ng) to previously extracted patient samples and observing increased peak height at the corresponding retention times.

The limit of detection (2/1 signal/noise) of this procedure for INH and ACINH is 0.10 ug/ml plasma. Repetitive injections of standards gave good reproducibility of retention times (standard deviation ± 1.5%) and peak heights (standard deviation ± 2.2%). Standard curves were linear in the range of 0.10 to 10 ug for INH and ACINH and the day to day reproducibility varied less than 2.7% (standard deviation).

The standard solutions for the three compounds were stable at least one week when stored at 4°C. Samples extracted from plasma decompose within 24 hours and refrigeration at 4°C or -15°C does not slow this process; therefore, all samples were analyzed within 4 hours following plasma extraction.

In Table 1 are given the levels (ug/ml plasma) of INH and ACINH after an oral dose of 300 mg INH. The drug peaks 1 to 2 hours after administration and the metabolite, in this patient, peaks within 3 to 4 hours. INH is still detectable 12 hours after dosing from a 2 ml plasma sample taken at the specified time.

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TABLE 1

Levels of Isoniazid (INH) and Acetyl-isoniazid (ACINH) in Human Plasma

Time* (Hours)	INH**	ACINH**
0	----	----
1	1.71 ± 0.51	0.30 ± 0.12
2	2.92 ± 0.72	1.41 ± 0.40
4	1.50 ± 0.50	2.09 ± 0.68
6	0.97 ± 0.43	0.61 ± 0.34
9	0.52 ± 0.33	----
12	0.25 ± 0.11	----

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* Time after an oral dose of 300 mg INH.

** Levels expressed as ug drug per ml plasma (average of three determinations) ± standard deviation.

In conclusion, the HPLC method described is sufficiently specific and sensitive for determination of INH and ACINH in human plasma following oral dosing of the drug.

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