Table I—Comparison of *In Vitro* and *In Vivo* Data [a]

Tablet	Percent Dissolved [b]		Plasma Prednisolone [c], ng/ml	
	10 min	20 min	30 min	60 min
1	83.3	91.5	162	231
2	54.1	64.9	108	186
3	89.6	92.4	162	225
4	56.4	67.4	113	172
5	87.7	94.5	162	244
6	87.2	95.5	164	222
7	14.1	51.2	22	79.7

[a] Manufacturers and lot numbers of the tablets were: 1, Nysco Laboratories, 49571; 2, Barr Laboratories, 4126111; 3, McKesson Laboratories, 3K668; 4, Rexall Drug Co., E11499; 5, Lemmon Pharmacal, 1382; 6, The Upjohn Co., 786AEFI; and 7, Danbury Pharmacal Inc., 4539. The products were submitted by the FDA National Center for Drug Analysis, St. Louis, Mo. [b] Average of three runs. [c] From Ref. 4, Table IV, p. 163, and Table IX, p. 170.

DISCUSSION

The peristaltic dissolution apparatus has provided the authors with complete assurance that a new product launched on the market is bioequivalent to an originator's product or formulated to present optimum drug release characteristics. This assurance has been demonstrated by the excellent *in vitro–in vivo* correlations obtained in beagle dog studies, comparative bioavailability evaluations in humans, and results from clinical studies.

The utility of the peristaltic apparatus in drug product design can be exemplified in the following manner. If the drug substance (*e.g.*, prednisone) has limited water solubility, a drug suspension is screened for dissolution characteristics in dilute hydrochloric acid solutions (0.1–0.001 *N*), distilled water, and pH 7.6 tromethamine buffer. This procedure is followed in the event that the drug exhibits polymorphism or possesses amphoteric characteristics and because of pH variations observed in gastric fluids (6). If the drug is available commercially, a minimum of six lots of the originator's product is subjected to the same dissolution treatment. Any tablet or capsule formulation developed in the laboratories then must comply with the dissolution profiles exhibited by the originator's product. This routine is followed through to production scaleup, and the first six production batches of a new product are monitored carefully by the peristaltic apparatus. Any formulation change including an increase in batch size is investigated for possible changes in drug release characteristics before the product is released for sale.

Additional *in vitro–in vivo* correlations obtained with the peristaltic dissolution apparatus and initial drug absorption profiles in beagles are under investigation.

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High-Performance Liquid Chromatographic Determination of Isoniazid and 1-Isonicotinyl-2-lactosylhydrazine in Isoniazid Tablet Formulations

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Abstract □ A high-performance liquid chromatographic procedure is presented for the simultaneous determination of isoniazid and 1-isonicotinyl-2-lactosylhydrazine (I) in isoniazid tablet formulations. An aliquot of a diluted aqueous tablet extract is introduced onto a microparticulate cyanopropyl bonded-phase column using a valve-loop injector and chromatographed using a mobile phase of acetonitrile–0.01 *M*, pH 3.5 aqueous acetate buffer (5:95). Compound I can be determined at levels as low as 0.5% of the isoniazid label claim. The relative standard deviations are 0.4 and 0.7% for the simultaneous determination of isoniazid and I, respectively. Seven commercial tablet formulations contained 93.8–97.0% of the labeled isoniazid amounts and 0.3–5.8% of I, expressed as equivalent isoniazid relative to the labeled isoniazid level.

Keyphrases □ High-performance liquid chromatography—simultaneous determination of isoniazid and 1-isonicotinyl-2-lactosylhydrazine, isoniazid tablet formulation analysis □ Isoniazid—high-performance liquid chromatography, isoniazid tablet formulation analysis, simultaneous determination with 1-isonicotinyl-2-lactosylhydrazine □ 1-Isonicotinyl-2-lactosylhydrazine—high-performance liquid chromatography, isoniazid tablet analysis, simultaneous determination with isoniazid

1-Isonicotinyl-2-lactosylhydrazine (I) has been reported in isoniazid tablets formulated with lactose (1, 2). The formation of I from the interaction of isoniazid and lactose is marked at high humidity and/or elevated temperatures (3). TLC of various isoniazid tablet formulations available in Canada showed appreciable levels of I. The USP XIX (4) nitrite titration assay for isoniazid responds quantitatively to the isoniazid moiety of I (5); therefore, an alternative procedure was required to monitor isoniazid formulations for I. Several colorimetric procedures (2, 6) are specific for isoniazid in the presence of I but cannot directly measure I.

A chromatographic method would be applicable to the rapid, simultaneous determination of isoniazid and I. TLC was unsuitable because of the difficulty in obtaining quantitative data, and GLC was rejected because of chromatographic difficulties with I. High-performance liquid chromatography (HPLC) has been used for isoniazid analysis in single- (1) and dual- (7) component formulations but has not been applied to the analysis of I except (1) to show that such compounds do not interfere with the isoniazid peak. A forward-phase HPLC system was developed recently for the simultaneous analysis of isoniazid and α- and β-1-glucopyranosyl-2-isonicotinylhydrazine

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in isoniazid sugar syrup formulations[1]. However, this system is not applicable to the determination of I in isoniazid tablet formulations because of the late elution of and low sensitivity for I.

This article describes the HPLC system developed for the quantitation of low levels of I and isoniazid in isoniazid tablet formulations.

EXPERIMENTAL

Reagents and Chemicals—Isoniazid[2] raw material was assayed before use by the USP (4) procedure (100.5%), and its purity was confirmed by HPLC. Compound I was synthesized from 0.2 mole of isoniazid and 0.2 mole of lactose[3] in 200 ml of water at room temperature (23 ± 1°). After 7 days, a white, crystalline product was precipitated by the addition of a small amount of methanol and cooling to 4°. The product was filtered, washed with acetone, and recrystallized from water-methanol until the HPLC[4] and TLC[5] tests for residual isoniazid and lactose, respectively, were negative. The product purity, mp 214-216° (uncorr.) [lit. (8) mp 202°], was determined by the isoniazid moiety titration using the USP XIX (4) nitrite titration procedure.

All other reagents and chemicals were commercial analytical reagent grade except for the distilled water, which was double distilled and filtered through a 0.2-μm filter[6] before use.

Chromatography—The liquid chromatograph consisted of a single-piston metering pump[7], a valve-loop injector[8] equipped with a 10-μl loop, a fixed-wavelength UV detector[9] [254 nm, 0.02 absorbance unit full scale (aufs)], a 10-mv strip-chart recorder[10] connected to the recorder output, and an electronic-computing integrator[11] connected to the detector absorbance terminals.

The mobile phase, aqueous 0.01 *M* acetate buffer (pH 3.5)–acetonitrile (95:5 v/v), was pumped through a 3.2 × 250-mm cyanopropyl bonded-phase column[12] at 1.5 ml/min (80 bar).

Calibration Curves—Aqueous stock solutions of isoniazid, 5.0 mg/ml, and of I, 0.125 mg/ml, were prepared. Standard solutions were prepared by pipetting 1, 2, 3, 5, or 10 ml of the isoniazid stock solution into each of five 50-ml volumetric flasks along with 1, 2, 5, 10, or 20 ml of the I stock solution. Each standard solution was diluted to volume with water, and duplicate 10-μl aliquots of each were chromatographed.

Calibration Standard Solutions—Compound I, ~5 mg, and isoniazid, 50 mg, were weighed accurately, transferred to a 100-ml volumetric flask, dissolved, and diluted to volume with distilled water (Solution A). A second calibration standard was prepared by diluting 5.0 ml of Solution A to 25.0 ml with water (Solution B). Duplicate 10-μl aliquots of each calibration standard were chromatographed periodically to check the slopes of the calibration curves.

Analysis of Pharmaceuticals—*Single-Tablet Assay*—A tablet was placed in a 20 × 150-mm screw-capped culture tube[13] and finely powdered with a glass rod. Sufficient distilled water was added to give a theoretical isoniazid concentration of 5.0 mg/ml, *e.g.*, 20.0 ml of water for a 100-mg tablet. The tightly capped tube was rotated[14] at 30 rpm for 15 min and then centrifuged[15] briefly at 1000 rpm. Five milliliters of the clear aqueous supernate was transferred to a 50-ml volumetric flask and diluted to volume with distilled water. Duplicate 10-μl aliquots of the resulting solution were chromatographed.

Tablet Composite Assay—A tablet composite was prepared by grinding 20 tablets in a mechanical mill[16]. A quantity of powdered tablet material equivalent to 50 mg of isoniazid was transferred to a 20 × 150-mm screw-capped culture tube, 10 ml of water was added, and the sample was centrifuged and chromatographed as described under *Single-Tablet Assay*.

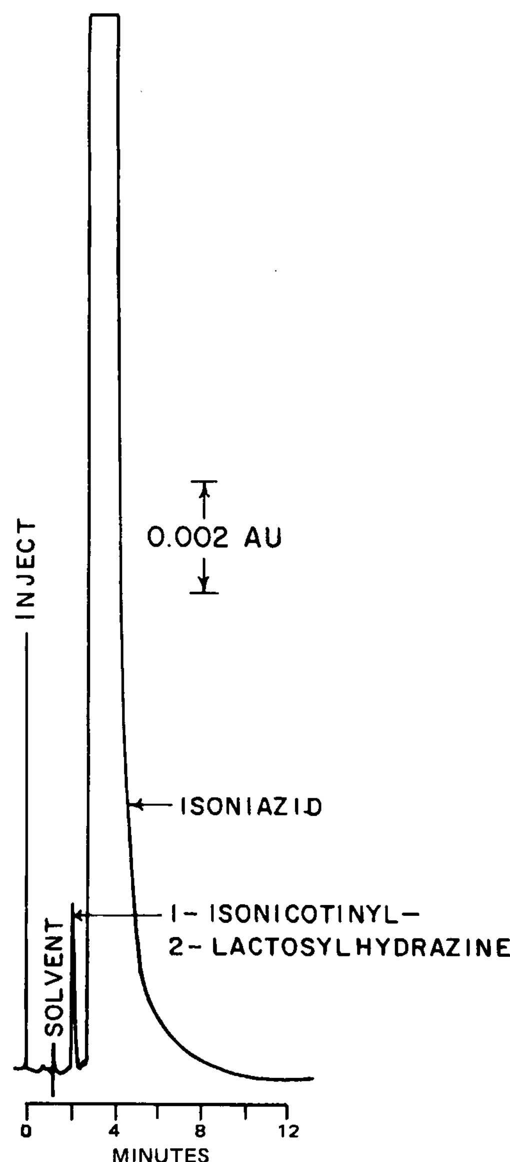

Figure 1—*High-performance liquid chromatogram of a typical isoniazid tablet extract.*

Quantitation—Quantitation was by comparison of the peak height for I and the peak area for isoniazid to the calibration standards and was based on absolute injection of 10-μl samples.

RESULTS AND DISCUSSION

Chromatographic System—Previous reports of HPLC systems for isoniazid analysis either did not describe the chromatographic behavior of I (7) or indicated a long retention time and, therefore, poor sensitivity (1). To achieve maximum sensitivity, reversed-phase HPLC was necessary to elute I ahead of isoniazid. Octadecyl bonded-phase columns[17] provided the desired elution order but gave retention times that decreased over a few days until both I and isoniazid eluted at the solvent front.

A cyanopropyl bonded-phase column[12] gave comparatively good results with the mobile phase described under *Experimental*. Satisfactory separations were obtained on three replicate cyanopropyl columns, each requiring somewhat different mobile phases. A simple water-acetonitrile

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[1] To be published.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] British Drug Houses, Toronto, Ontario, Canada.
[4] Conditions as described under *Experimental*.
[5] TLC conditions were silica gel GF 60 (Merck), 0.25 mm with a developing solvent of acetone-methanol-acetic acid (50:50:10). The R_f values were: lactose, 0.31; isoniazid, 0.42; and 1-isonicotinyl-2-lactosylhydrazine, 0.20. UV visualization was at 254 nm after spraying with *p*-methoxybenzaldehyde-ethanol-sulfuric acid (1:18:1) and heating at 100° to show the lactose spot.
[6] FGLP 04700, Millipore Ltd., Mississauga, Ontario, Canada.
[7] Model 110A, Altex Scientific, Berkeley, Calif.
[8] Model CV-6-UHPa-N60, Valco Instruments Co., Houston, Tex.
[9] Model 440, Waters Associates, Milford, Mass.
[10] Model 56, Perkin-Elmer Corp, Norwalk, Conn.
[11] System I, Spectra-Physics, Santa Clara, Calif.
[12] Spherisorb CN (5 μm), Altex Express Series, Altex Scientific, Berkeley, Calif.
[13] Canadian Laboratory Supplies, Montreal, Canada.
[14] Multipurpose rotator, Scientific Industries, Springfield, Mass.
[15] Model K, International Equipment Co., Needham Heights, Mass.
[16] Micro mill, Chemical Rubber Co., Cleveland, Ohio.
[17] RP18, E. Merck, West Germany (through British Drug Houses, Toronto, Canada).

Table I—Retention Times for Isoniazid and Various Related Compounds

Compound	Retention Time[a], sec
Isoniazid	258
1-Isonicotinyl-2-lactosylhydrazine (I)	133
Isonicotinic acid	75[b]
Isonicotinamide	184
1-Isonicotinyl-2-glucosylhydrazine (II)	128
1-Isonicotinyl-2-galactosylhydrazine (III)	130
4-Aminosalicylic acid	79
Pyridoxine hydrochloride	>900

[a] The column was a 3.2 × 250-mm Spherisorb CN (5 μm), the flow rate was 1.5 ml/min, and the mobile phase was 5% acetonitrile in aqueous 0.01 *M* sodium acetate buffer (pH 3.5). [b] Solvent front (t_0).

(95:5) mobile phase initially eluted isoniazid as a retained peak, but retention decreased to zero over a few days. Substitution of a dilute aqueous acetate buffer for water in the mobile phase stabilized the retention time at a value dependent on the buffer pH and concentration. Increased retention times were obtained as the pH or ionic strength was decreased. Since retention times also increased as the acetonitrile proportion in the mobile phase was reduced, the separation mode can be described as reverse phase.

After several weeks, the retention times for isoniazid and I decreased to an unacceptable degree. The column was regenerated by pumping 1% aqueous HCl at 1.5 ml/min for 1 hr. On returning to the original mobile phase conditions, this procedure initially led to much longer retention times followed by a slow return to the original separation.

Figure 1 shows a chromatogram obtained when a representative tablet formulation was analyzed. The compounds were eluted in less than 12 min, a major portion of this time being necessary for the elution of the long tail on the isoniazid peak.

Various isoniazid-related compounds were chromatographed (Table I), either because of their possible occurrence as impurities or because they are sometimes formulated with isoniazid in two-component tablets. 1-Isonicotinyl-2-glucosylhydrazine (II) and 1-isonicotinyl-2-galactosylhydrazine (III) may be present in tablet formulations through isoniazid interaction with lactose hydrolysis products, glucose and galactose. Since I–III are not resolved, the procedure is not specific for I in the presence of these impurities. However, since the three compounds exhibited the same molar detector response, due entirely to the isoniazid moiety, total isoniazid in the form of sugar hydrazones may be determined by quantifying the peak at the retention time of I.

Linearity and Standard Curves—The isoniazid standard curve (peak area *versus* amount injected) was linear with a negligible intercept ($y = mx$) over 1.0–10.1 μg/injection (0.1–1.10 mg/ml). Similarly, the I standard curve (peak height *versus* amount injected) was linear with a negligible intercept ($y = mx$) over 25–500 ng/injection (2.5–50 μg/ml). Therefore, the standard curves are applicable to tablets containing 20–200% of the isoniazid label claim level and 0.5–10% of I (or 0.15–3.0% of I expressed as equivalent isoniazid).

Response factors were determined by two or more injections of two calibration solutions at the start of each day or whenever the mobile phase was replenished. Agreement of the response factors within 3% at the two levels was considered adequate but was typically better than 2%. After checking the response at two levels to show linearity, a single calibration standard was chromatographed periodically to check for response changes. Due to gradual hydrolysis of I, calibration standards must be prepared daily.

Sample Preparation—The time required for constant extraction of isoniazid and I from the tablet mass was determined by tumbling crushed single tablets of a representative formulation, 100 mg, for 5, 10, 15, 20, and 30 min in 20 ml of water. Each sample was centrifuged briefly at 1000 rpm, and 1.0 ml was diluted to 10.0 ml with water. Duplicate 10-μl aliquots of each diluted sample were chromatographed. The peak heights for I and the peak areas for isoniazid were the same in all samples, indicating constant extraction of both compounds in less than 5 min. Based on response factor data and the label claim for the formulation used, extraction also appeared to be complete, and a geometric dilution procedure substantiated this finding. When tablet composite samples equivalent to one-half, one, and two tablets were analyzed (15-min tumbling time), the I and isoniazid concentrations were both in the ratio 1:2:4, showing that complete extraction took <15 min.

Table II—HPLC Analysis of Isoniazid Tablet Formulations[a]

Formulation	Label Claim, mg	Compound I[b,c], %	Isoniazid, % label claim[b]
A	50	0.39	93.9
B	300	ND[d]	95.9
C	100	0.35	96.8
D	100	0.31	94.4
E	100	5.81	93.8
F	100	0.29	96.3
G	100	0.96	97.0

[a] Tablet composite assay. [b] Mean of duplicate assays. [c] Based on the isoniazid moiety of I, *i.e.*, isoniazid equivalent. [d] None detected.

Quantitation—Samples, 10 μl, were introduced *via* a valve-loop injector using the loop-fill mode. This absolute injection technique gave precise, accurate results and eliminated the need for an internal standard. Initially, peak area was used for both compounds; but during the procedure testing, the separation of isoniazid and I on different columns was usable over a wider resolution range when peak height was used for quantitation of I. Ten injections of a representative formulation extract gave relative standard deviations of 0.7 and 0.4% for I and isoniazid, respectively.

Analysis of five replicate composite samples of a representative isoniazid tablet formulation containing 3.3% of I (0.96% expressed as equivalent isoniazid) and 97.0% of the isoniazid label claim level gave relative standard deviations of 1.8 and 0.9%, respectively.

Seven commercial formulations were analyzed in duplicate (Table II). In tablets formulated with lactose, the I levels varied between 0.3 and 5.8%, expressed as equivalent isoniazid, while isoniazid levels varied between 93.8 and 97.0% of the label claim.

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