Quantitative Determination of Isosorbide Dinitrate and Two Metabolites in Plasma

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Abstract □ A GLC method for the determination of plasma isosorbide dinitrate and two metabolites, isosorbide 2-mononitrate and isosorbide 5-mononitrate, is described. The three substances are extracted from alkalinized plasma with ether. Quantitation is effected by electron-capture detection after GLC separation with a 30% SE-30 column. The unusually heavy liquid phase loading is necessary to eliminate irreversible adsorption on the solid support. The electron-capture detector provides excellent sensitivity and specificity because of the electronegative nature of the nitric ester. The method was used to study the blood levels of isosorbide dinitrate and two metabolites in four beagle dogs after single oral doses of 40 mg of isosorbide dinitrate and in two human volunteers after a sublingual dose of 10 mg of isosorbide dinitrate.

Keyphrases □ Isosorbide dinitrate—and metabolites, electron-capture GLC analysis, plasma □ GLC, electron capture—analysis, isosorbide dinitrate and metabolites, plasma □ Vasodilators, coronary—isosorbide dinitrate and metabolites, electron-capture GLC analysis, plasma

Nitric esters of various polyols are used in the treatment of angina pectoris (1). Isosorbide dinitrate (1,4:3,6-dianhydro-D-glucitol 2,5-dinitrate) (I) is widely used as a coronary vasodilator. The purpose of this work was to develop a method that would yield quantitative data for I and two metabolites, isosorbide 2-mononitrate (II) and isosorbide 5-mononitrate (III).

TLC was used to study levels of I–III in biological fluids (2, 3), and ^{14}C-labeled I was utilized to study its metabolism in dogs (4, 5). A GLC method using a flame-ionization detector was developed to determine I in plasma (6), and it was found that the previously reported TLC procedures (5) did not give the required sensitivity. GLC with electron-capture detection appeared to be the best methodology available for analysis of plasma samples.

An electron-capture GLC method (7) detected I–III within the same chromatogram; subsequently, this method was applied to plasma samples (8). Unfortunately, even with an elaborate cleanup, extracts of blank plasma sometimes yielded high background responses that interfered with the assay of II and III.

The goal of this work was to develop a more sensitive and convenient method that would lend itself to the analysis of a large number of plasma samples. A very simple and rapid extraction without further purification of the extract was feasible if the electron-capture GLC system was capable of detecting 150 pg each of I, II, and III. None of the previously described electron-capture GLC procedures had the necessary sensitivity for all three substances, apparently because of the irreversible interaction of these substances with the partially exposed solid support. The desired sensitivity was obtained when the active sites on the solid support were reduced through the use of a packing with 30% SE-30, an unusually high percentage of liquid phase. The result was an order of magnitude increase in sensitivity over previously described procedures for II and III (7, 8).

This methodology was used for the analysis of dog and human plasma after administration of I. Plasma I–III concentrations were determined after single oral doses of 40 mg of I. Plasma I–III levels also were measured in two human volunteers after a sublingual dose of 10 mg of I.

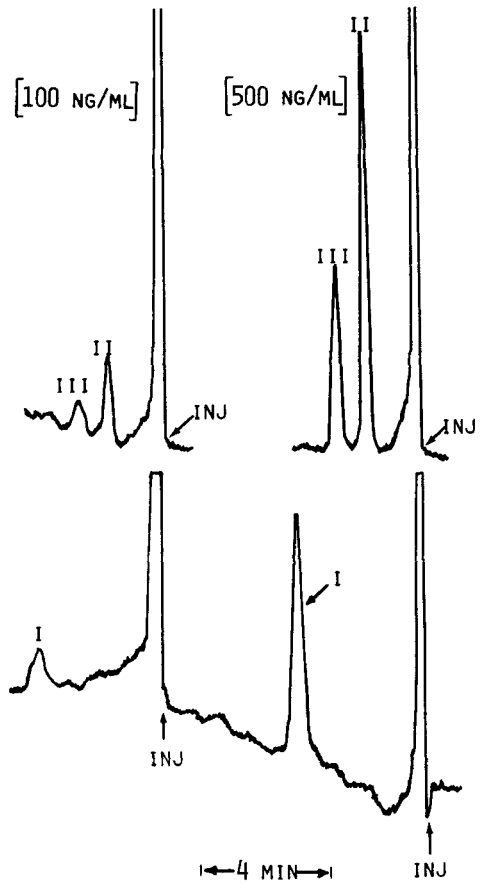

Figure 1—*Typical gas chromatographic results derived for I–III. Injections of 3 μl were used. The figures in brackets correspond to the concentrations of the stock solution.*

EXPERIMENTAL

Apparatus—A gas chromatograph[1] equipped with a ^{63}Ni-electron-capture detector was used. The carrier gas had a flow rate of ~60 ml/min. A silanized glass column, 2 mm i.d. × 1.83 m, was packed with 30% SE-30 on 60–80-mesh Gas Chrom Q[2]. Injection port, column, and detector temperatures were maintained at 190, 165, and 250°, respectively. Injections of 2–3 μl were made with a 10-μl syringe[3]. The preparation of the column packing must be done carefully to ensure good chromatographic results.

Reagents and Chemicals—Authentic samples of II and III were synthesized[4]. Compound I was purified by recrystallization from ethanol.

[1] Microtek MT-220 or Varian model 2100-20.
[2] Applied Science Labs, State College, Pa.
[3] Hamilton Co., Reno, Nev.
[4] By Dr. B. Palameta, Ayerst Research Laboratories, Montreal, Quebec, Canada.

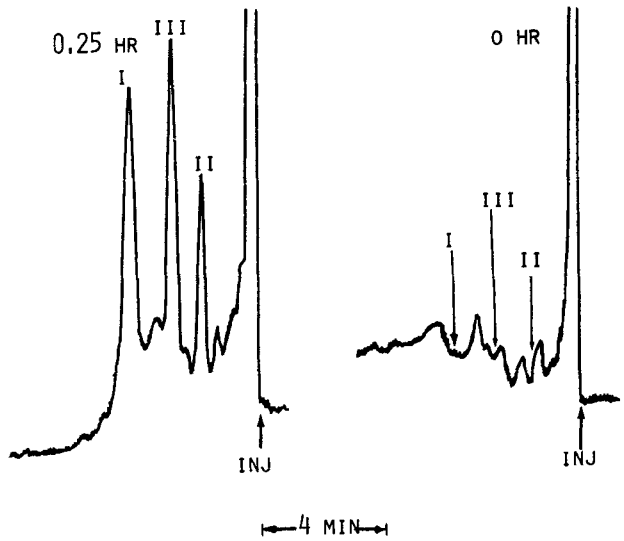

Figure 2—*Results obtained after oral administration of 40 mg of I to a dog. The 0.25-hr levels correspond to I, II, and III levels of 80, 41, and 120 ng/ml, respectively.*

The purity was 99.9% by differential scanning calorimetry. All other reagents were reagent grade except ethyl acetate, which was pesticide quality[5].

Determination of I–III in Plasma—To a 3-ml sample of dog plasma or to 4 ml of human plasma in a 50-ml centrifuge tube, equipped with a polypropylene stopper, was added 2 ml of 1 *N* NaOH or 1 ml of 2 *N* NaOH, respectively. Then 30 ml of ether was added, and the tube was stoppered and shaken mechanically[6] at low speed for 20 min. It was centrifuged at 1500 rpm for 5 min, and the aqueous layer was discarded. Then 0.5 g of anhydrous magnesium sulfate was added, and the mixture was shaken and centrifuged.

A 25-ml aliquot of the ether layer was transferred to a 30-ml centrifuge tube and evaporated to dryness under a gentle stream of nitrogen with the water bath held below 30°. The residue was redissolved in 1.0 ml of ethyl acetate. An aliquot (1–3 μl) of this solution was injected and compared with the calibration curve.

Calibration Curve—Calibration was done with the external standard procedure. Separate solutions of I–III were prepared in ethyl acetate at three different concentrations ranging from 100 to 500 ng/ml. The response as a percentage of the total standing current for each substance was plotted as a function of concentration.

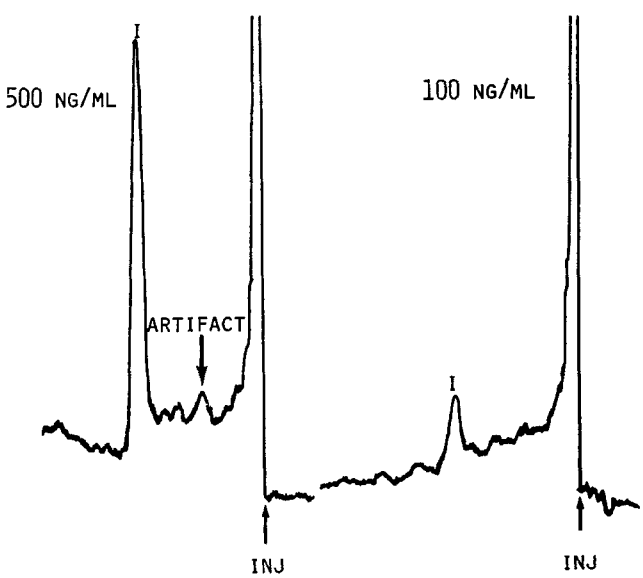

Figure 3—*Chromatograms illustrating artifact formation with exhausted column.*

[5] Fisher.
[6] Eberbach Corp.

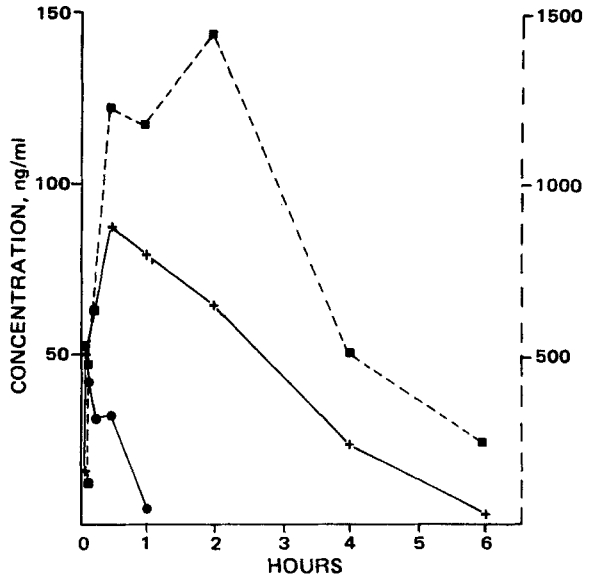

Figure 4—*Average plasma levels of I (●), II (+), and III (■, right-hand scale) in four beagle dogs after an oral administration of 40 mg of I.*

***In Vivo* Studies**—Beagle dogs, 10–12 kg, were given a single oral dose of 40 mg of I (eight 5-mg tablets[7]) after an overnight fast. Water was allowed *ad libitum*, and food was withheld until completion of sampling. Blood samples (7 ml) were collected in tubes[8] with edetate disodium at premedication and at 0, 5, 10, 15, 30, 60, 120, 240, and 360 min postmedication. Samples were centrifuged, and the plasma was separated and stored in a freezer until analysis.

In a second study, two human volunteers were given two 5-mg sublingual tablets[9]. Blood samples were taken using tubes[8] with edetate disodium at 0, 10, 20, 30, 60, 120, and 240 min postmedication. Plasma was separated and frozen until assayed.

RESULTS AND DISCUSSION

Typical gas chromatograms for I–III are shown in Fig. 1. The false positive levels were determined for 16 different samples of dog plasma and were equivalent to less than 2 ng/ml for I and II. A peak often interfered with III, but the magnitude never exceeded 5 ng/ml, far below the peak levels ordinarily observed for medicated dogs.

The chromatograms illustrated in Fig. 2 are typical of the results obtained after oral administration of I. The response of the electron-capture detector to I–III was linear with concentration over the 50–1000-ng/ml

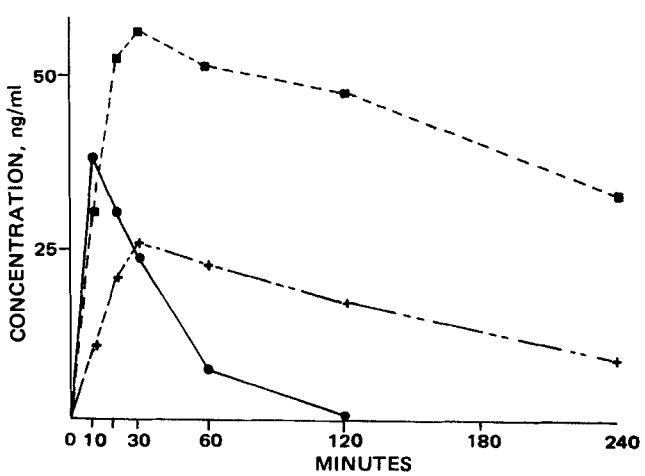

Figure 5—*Average plasma levels of I (●), II (+), and III (■) in two human volunteers after sublingual administration of 10 mg of I.*

[7] Isordil, Laboratories Ayerst LTDA, Sao Paulo, Brazil.
[8] Vacutainers.
[9] Isordil, Ives Laboratories, New York, N.Y.

Table I—Recoveries of I–III from Plasma

Compound	Dog Plasma			Human Plasma		
	n	Recovery, %	*CV*	*n*	Recovery, %	*CV*
I	7	83	7	16	67	10
II	8	59	9	16	65	8
III	8	71	12	16	59	16

range, having a standing current of 4.7×10^{-9} amp in the pulse mode with a width of 5 μsec at 44 v and a pulse interval of 220 μsec.

The partition ratios for I, II, and III between ether and physiological saline proved to be ~6, 0.5, and 0.35, respectively. The recovery from spiked samples for each substance is given in Table I.

After considerable usage, a column that otherwise appeared to be performing satisfactorily gave rise to an artifact upon injection of I. This artifact had the same retention time as II (Fig. 3). For this reason, the I standard was chromatographed at the beginning, middle, and end of the day without the two isosorbide mononitrates. The column was discarded as soon as the artifact peak was detected; however, additional useful column life was obtained by removing the top 5 cm of packing from the inlet end of the column and replacing with new conditioned packing. Under continuous use, a column lasted for approximately 1 month.

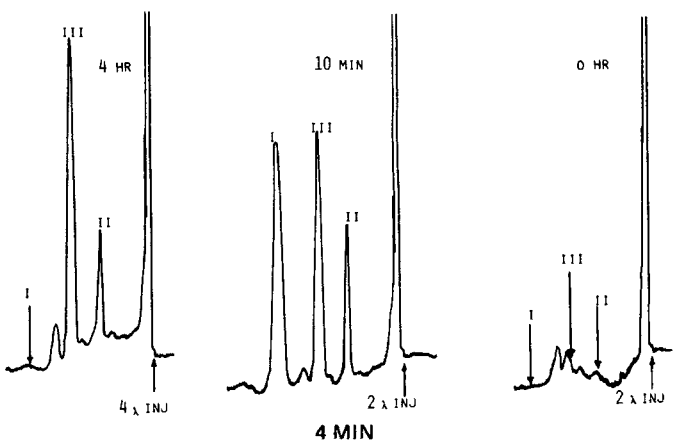

Figure 6—*Chromatograms illustrating the determinations of I–III in human plasma after sublingual administration of 10 mg of I.*

The average plasma I–III levels in four dogs are shown in Fig. 4. The results are in qualitative agreement with the data obtained in a ^{14}C-labeled study (4). There were very low and erratic blood levels of I, consistently higher levels of II, and much higher and prevalent levels of III. Very rapid absorption of I took place, since I levels were detected at 5 min postadministration.

The average plasma levels of I–III in two human volunteers are depicted in Fig. 5. Significant I levels were detected at 10, 20, and 30 min and up to 1 hr postadministration. The two metabolites reached a maximum at 30 min, and the III levels were approximately twice the II levels. Typical chromatograms are presented in Fig. 6.

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Selective Photometric Determination of Betamethasone Benzoate and Other 21-Hydroxycorticosteroids

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Abstract □ Condensation of the glyoxal obtained by cupric acetate oxidation of 21-hydroxycorticosteroids with acetous phenylhydrazine reagent affords a near UV chromophore. All of the tested corticosteroids, including triamcinolone acetonide, which gives low color yields in the Porter–Silber reaction and its Lewbart–Mattox modification, gave similar absorption maxima (362–370 nm) and molar absorptivities (ϵ = 17,000–20,500). Since corticosteroid 21-esters and oxidation products do not undergo the reaction, the assay method based on it is stability indicating for betamethasone benzoate and the other test compounds. Procedures are described for the assay of two topical betamethasone benzoate preparations and hydrocortisone and prednisolone tablets; recovery and precision data are given.

Keyphrases □ Betamethasone benzoate—UV photometric analysis, bulk drug and pharmaceutical formulations □ UV photometry—analysis, betamethasone benzoate and other 21-hydroxycorticosteroids, bulk drug and pharmaceutical formulations □ 21-Hydroxycorticosteroids, various—UV photometric analysis, bulk drug and pharmaceutical formulations □ Glucocorticoids, various—UV photometric analysis, bulk drug and pharmaceutical formulations

The Porter–Silber reaction (1) for the colorimetric determination of corticosteroids is applicable only to steroids with a 17-dihydroxyacetone side chain ($COHCOCH_2OH$) or their derivatives that are readily hydrolyzed in the strongly acid reaction medium. Its mechanism (2, 3) was elucidated as a two-stage reaction: acid-catalyzed rearrangement of the side chain to a 17-deoxy-20-one-21-al ($CHCOCHO$), followed by condensation of this glyoxal