input. This is true for the examples involving exponential drug input functions cited by Chiou (Tables I–IV in Ref. 1). Since the point–area method defines a staircase input function, the method gives exact results for zero-order absorption rates. Consequently, the examples cited by Chiou for zero-order absorption (Tables VI and VII in Ref. 1) simply reflect the degree of accuracy of the approximations, specified by Eq. 4, for the particular characteristic responses used in the examples. When the approximations of Eq. 4 are invalid, the instantaneous midpoint–input method gives erroneous cumulative drug input functions. As an example, consider the plasma concentrations (micrograms per milliliter) of lidocaine after a 1-mg iv bolus as the characteristic response, where $G(t) = 0.0276e^{-0.123t} + 0.0084e^{-0.00673t}$ and the units of t are minutes.

Apply the instantaneous midpoint–input deconvolution method to the plasma drug concentrations that would result from a 1.86-mg/min constant intravenous infusion of lidocaine. The results of applying this method are given in Table I. This example illustrates the large errors that can result by approximating integrals by rectangular functions. The point–area method gives exact results for this example (Table I).

In conclusion, the instantaneous midpoint–input method is an approximation of the point–area method. The use of approximations in the latter method can result in large errors in the cumulative drug input functions, and such unnecessary approximations should be avoided.

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Stability of Nitroglycerin Ointment

Keyphrases □ Nitroglycerin—stability of ointment at elevated temperatures □ Vasodilators—stability of nitroglycerin ointment at elevated temperatures □ Stability—nitroglycerin ointment at elevated temperatures

To the Editor:

Topical nitroglycerin therapy offers a longer duration of action than sublingual administration and thus is popular as a prophylactic treatment for angina pectoris (1, 2). Previous studies indicated that the potency of nitroglycerin tablets may be affected by the environment in which the tablets are stored (3, 4). It also was demonstrated that nitroglycerin in solutions for intravenous use interacts with plastic infusion bags and intravenous giving sets (5, 6), resulting in decreased availability of nitroglycerin. Since we are unaware of any report on the stability of nitroglycerin ointment during storage, we evaluated the stability of nitroglycerin ointment capsules stored at elevated temperatures for extended periods.

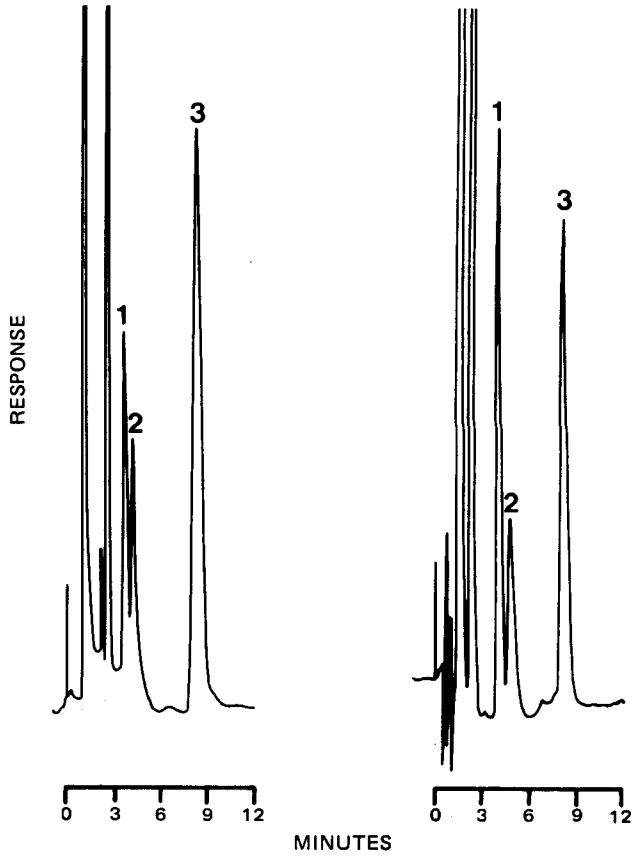

Figure 1—*(Left) Chromatogram of an aqueous solution of nitroglycerin and the two dinitroglycerins. Key: 1, 1,3-dinitroglycerin; 2, 1,2-dinitroglycerin; and 3, nitroglycerin. (Right) Chromatogram of an octanol extract of nitroglycerin ointment. Key: 1, unknown interfering peak; 2, unknown interfering peak; and 3, nitroglycerin.*

Capsules from unopened bottles of nitroglycerin (3% w/w) ointment capsules[1] were stored on individual glass dishes in an oven at 37 ± 1° and in the original glass bottle at room temperature (20–24°) in the dark. Capsules were removed from storage after 5, 10, and 18 weeks and were assayed for nitroglycerin content as follows. Each capsule was cut open, the ointment was removed completely, and its weight was recorded. The nitroglycerin then was extracted from the ointment by vortexing with 5 ml of octanol for 10 min and centrifuging at 3000 rpm for 10 min. This method achieved >96% extraction of nitroglycerin.

The extract then was diluted with methanol before assay using kinetic (7) and high-performance liquid chromatographic (HPLC) methods (8). Standard curves for the two methods were constructed by adding known amounts of nitroglycerin, standardized by the method of Dean and Baun (9), to octanol, which then was diluted in methanol to give appropriate concentrations for analysis. In four determinations of six different nitroglycerin concentra-

[1] Nitrolate, Roche Products, Sydney, Australia.

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Table I—Percentage of Nitroglycerin in Ointment Capsules Stored at 37° for 18 Weeks

Weeks	Percent Content (Mean ± *SE*, *n* = 5) Kinetic Assay	HPLC Assay
0	3.24 ± 0.06	3.19 ± 0.11
5	3.18 ± 0.10	3.23 ± 0.08
10	3.17 ± 0.12	3.20 ± 0.10
18	3.22 ± 0.05	3.19 ± 0.09

tions, the correlation coefficient of results obtained by the two methods was 0.988.

Unfortunately, the HPLC method and the other previously reported high-pressure liquid chromatographic method (10) were not specific for glyceryl dinitrates due to the presence of unknown interfering peaks from the ointment extract (Fig. 1). No reduction in size of the interfering peaks was achieved by using other organic solvents (hexane, ether, and methanol) to extract the ointment. The relative height of these peaks did not change over time. It was established by TLC separation (11) prior to HPLC analysis that the amount of glyceryl dinitrates present in the ointment was negligible. Triacetin, which is used as a solvent for nitroglycerin in some preparations, cochromatographed with glyceryl 1,2-dinitrate using the method of Crouthamel and Dorsch (8).

No loss of nitroglycerin was found after storage of individual capsules for 18 weeks at 37° (Table I). The amount of the dinitrates present in the ointment was negligible throughout the study.

The good stability of nitroglycerin in the ointment formulation tested is consistent with a high affinity of nitroglycerin for the lipophilic ointment base and a low affinity for the gelatin capsule in which the ointment is presented. In contrast, the availability of nitroglycerin from aqueous solutions infused from plastic infusion bags through giving sets is reduced due to the high affinity of nitroglycerin for the plastic (12).

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Comparison of Plasma and Urine Analyses for Thiazides in Bioavailability/Bioequivalence Study

Keyphrases □ Thiazides—plasma *versus* urine analysis in bioavailability/bioequivalence study □ Bioavailability—thiazides, plasma *versus* urine analysis □ Bioequivalence—thiazides, plasma *versus* urine analysis □ Diuretics—thiazides, plasma *versus* urine analysis in bioavailability/bioequivalence study

To the Editor:

Thiazides are widely used as diuretics in the treatment of hypertension. In the recent proposal (1) for bioavailability/bioequivalence requirements, the thiazides were identified as having potential bioequivalence problems. To ensure bioequivalence of thiazide products, both *in vitro* dissolution and *in vivo* bioavailability requirements were proposed. The *in vivo* requirements call for bioavailability studies in humans and comparison of blood level and/or urinary excretion profiles of the drug with a standard reference product. It is generally assumed that blood (or plasma or serum) level measurements give a better assessment of bioavailability and bioequivalence than urinary measurements because of complicated pharmacokinetic considerations such as drug metabolism and urine collection problems.

It is sometimes argued that the presence of drug in the blood is not a real estimate of the drug availability at the site of action but only an estimate of drug bioavailability. It is preferable to determine the amount of drug at the site of action or to measure therapeutic effect, but these two procedures are generally not feasible. Thiazide diuretics are weak carbonic anhydrase inhibitors that enhance the renal excretion of sodium and chloride ions and an accompanying volume of water, thereby causing diuresis. This therapeutic effect is due to inhibition of ion reabsorption in the distal tubule. Thiazides are primarily excreted unchanged in urine by active secretion in the proximal tubule (2). Thus, from a pharmacological and a therapeutic viewpoint, measurement of the urinary excretion of the drug appears to be a logical choice in lieu of measurement in blood for the assessment of bioavailability of a diuretic dosage form.

Previous work on chlorothiazide and hydrochlorothiazide suggested that urinary level measurements give adequate information for bioavailability/bioequivalence assessment (3, 4). Figure 1 shows the amount of drug eliminated in the urine (AE) and the area under the curve (AUC) in the same subjects for various thiazides. For hydrochlorothiazide, ~34% of the administered drug was recovered in 0–12-hr urine samples at all dose levels (100, 50, and 25 mg), thus suggesting a dose–response relationship. As expected, the amount of drug in urine decreased with a decrease in hydrochlorothiazide administration, but the AUC did not correspond and correlate with the AE values (Subjects 2 and 3, Fig. 1A). Earlier data (5) for hydrochlorothiazide showed no relationship between the AUC and AE (Fig. 1B), between the AUC and product bioavailability, or between C_{max} and dose administered or bioavailability.

A preliminary study using chlorothiazide products showed that, although approximately the same amount of drug was excreted in 48 hr from subjects administered 250-

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