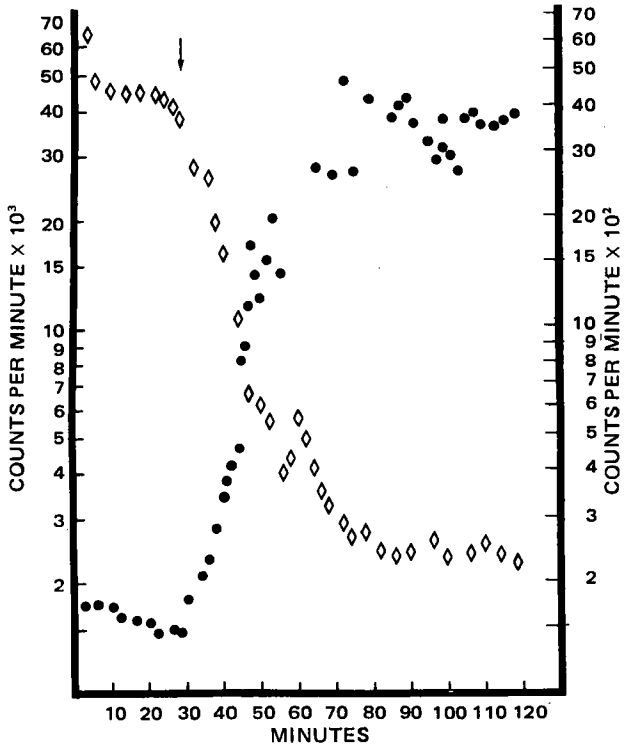

Figure 3—*Graphic display illustrating release of radioactivity from a gelatin capsule in the human stomach using Formulation A (empty stomach). Key: ◊, disappearance of radioactivity from the capsule region (Area 1, Fig. 2); and ●, concomitant appearance of radioactivity in the pyloric region of the stomach.*

interest in the lower pyloric region of the stomach (Fig. 2). Continuous monitoring of the radioactivity in the two regions, as a function of time, showed a sharp decrease in radioactivity in the capsule region, marking the time of its collapse, with a concomitant increase at the pyloric region. A typical plot is shown in Fig. 3. Further follow-up (Fig. 3) on the rate of passage of radioactivity through the lower stomach provided data for the estimation of gastric emptying times (1).

When the Formulation A capsule was administered to two subjects on an empty stomach, the scintiphotos and the integrated computer plots showed that the release of the capsule contents began 30 (Subject 1, Fig. 3) and 40 (Subject 2) min after ingestion. On a full stomach, the same two subjects exhibited much longer times for initial release of the contents, 93 and 120 min, respectively. In all cases, the capsule remained stationary and the capsule contents dispersed immediately to the other regions of the stomach after the initial release.

Upon administration of the Formulation B capsule to Subject 1 on an empty stomach, the initial release of radioactivity occurred after only 6 min. Considerable swelling of the capsule was also observed before the release. The decrease of activity from the capsule region was gradual up until 56 min after administration. At this point, there was a sharp break in the curve, indicating virtually complete disappearance of radioactivity from the capsule region.

The observed differences in the times of release of Formulations A and B from identical capsules can be explained on the basis of the differences in these formulations. The relatively large (40–100 mesh) insoluble resin particles of Formulation A can be dispersed into the stomach only after major collapse of the capsule walls, a rather lengthy process. But the water-soluble Formulation B can be released in a shorter time because the gastric juices gain access to the interior of the capsule *via* a small orifice or diffusion through the walls. These findings are consistent with the previously reported observation that increasing the water-soluble nature of a formulation will increase its rate of release from a gelatin capsule (2–4). It is also known that particle size and type of diluents or fillers used in various formulations can drastically affect the rate of their release from gelatin capsules (5).

In conclusion, this technique can provide valuable information regarding the *in vivo* behavior of capsules. The present method is noninvasive and exposes the subjects to an extremely low radiation dose. If the experiment is conducted with 20 μCi of technetium Tc 99m, the radiation dose to the whole body is 1 mrad and that to the stomach is 10 mrads (1). The present study illustrates the great potential of external scintigraphy in the assessment of drug formulations.

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High-Pressure Liquid Chromatographic Determination of Polythiazide in Pharmaceutical Dosage Forms

Keyphrases □ Polythiazide—high-pressure liquid chromatographic analysis, pharmaceutical dosage forms □ High-pressure liquid chromatography—analysis, polythiazide in pharmaceutical dosage forms □ Diuretics—polythiazide, high-pressure liquid chromatographic analysis in pharmaceutical dosage forms

To the Editor:

A recent report by Moskalyk *et al.* (1) utilized a high-pressure liquid chromatographic (HPLC) method

Table I—Comparative Analyses of Three Commercially Available Polythiazide Tablet Preparations

Product	Components	Analyses, % of Label Claim: Method of Moskalyk *et al.* (1)	Analyses, % of Label Claim: Method of Wong *et al.*[a] (3)
I[b]	Polythiazide, 1 mg	110	102
II[b]	Polythiazide, 2 mg	108	101
III[c]	Polythiazide, 1 mg, and reserpine, 0.25 mg	112	106

[a] Average of four determinations. [b] Renese, Pfizer Co. Ltd. [c] Renese-R, Pfizer Co. Ltd.

for the determination of polythiazide in pharmaceutical dosage forms. Three independent methods were used to analyze polythiazide tablets[1] (1): TLC separation followed by a UV spectrophotometric determination (2), a colorimetric method (2), and the published HPLC method (1). The results obtained by Moskalyk *et al.* (1) with their method and the compendial TLC method indicated that the polythiazide tablets were superpotent, exceeding the NF XIII limit of 110% of label claim (2). We were concerned with this implication and initiated a detailed study of the published method.

The column[2] recommended by Moskalyk *et al.* (1) was conditioned with the mobile phase, methanol–water (35:65 v/v), for at least 16 hr at 100 psig inlet pressure. The inlet pressure of the liquid chromatograph[3] then was raised to 200 psig, the reported operating pressure (1). A standard solution of polythiazide and the internal standard, quinoline, was introduced into the chromatograph using a 10-μl loop injection valve; this solution was prepared in accordance with the proposed HPLC procedure (1).

The observed polythiazide peak at a detection wavelength of 254 nm was sharp and symmetrical, having a retention time of approximately 8 min *versus* the 7 min reported previously (1). No peak was observed at the retention time reported for the internal standard. Additional column conditioning (total time of 4 days) and injections did not improve the peak characteristics or detector sensitivity for the internal standard. Increasing both the quinoline concentration from the reported 230 mg/liter to 1 g/liter and the inlet pressure to 600 psig resulted in an observed peak for quinoline at a retention time of approximately 17 min *versus* the reported 10 min at 200 psig (1). The peak was extremely broad and tailed considerably. We could not reproduce the reported observations for the internal standard.

Figure 2 in Ref. 1 does not exhibit baseline separation for polythiazide and the suggested internal standard, quinoline. In our opinion, this separation is a necessity in the establishment of a quantitative analytical procedure. Also, chromatogram III and, possibly, chromatogram II in Fig. 2 show the presence of an unknown absorption between vanillin and polythiazide. This absorption is illustrated by the shoulder on the vanillin peak and the lack of return to the minimum detector response observed in chromatogram I. Table III in Ref. 1 does not agree with our experience with the compendial method (2). The investigators (1) stated that erratic results were obtained with the NF XIII and colorimetric methods for the quantitation of polythiazide in combination with reserpine. We encountered no such interference due to reserpine in either method.

The most significant point concerns the recovery studies. The authors (1) stated that the absolute recovery of added polythiazide was 0.212 ± 0.003 mg based on four replicate determinations. They also stated that the added polythiazide was only 0.20 mg. A method in which published recoveries of 106% are presented without further explanation cannot be used with confidence. Results with such a method must be considered biased high.

At the time the article by Moskalyk *et al.* (1) appeared, research was already underway in our laboratories to develop an HPLC method for polythiazide in pharmaceutical tablet formulations. A successful assay has been developed and is described in a separate publication (3). In an effort to justify our criticism of the published method (1), we analyzed the same products. It was ascertained which batches of each product were donated to Moskalyk *et al.* (1), and our samples were taken from the same source. Results obtained by our HPLC method (3) are presented in Table I and are compared with those of Moskalyk *et al.* If the results of Moskalyk *et al.* (1) are corrected for the reported 6% high bias, their data are in excellent agreement with the results obtained by our method (3).

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[1] Renese and Renese-R, Pfizer Co. Ltd.
[2] Bondapak phenyl/Corasil, Catalog No. 27283, Waters Associates.
[3] DuPont model 820.

Ciguatera I: Brine Shrimp (*Artemia salina* L.) Larval Assay for Ciguatera Toxins

Keyphrases □ Ciguatera toxins—bioassay using brine shrimp larvae □ Fish toxins—ciguatera, bioassay using brine shrimp larvae □ Toxins, ciguatera—bioassay using brine shrimp larvae □ Bioassays—screening for ciguatera toxins using brine shrimp larvae

To the Editor:

No fully satisfactory bioassay for ciguatera toxins, a major problem for tropical and subtropical reef fisheries, has been described (1). Individual fish of most reef