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-$\mu$l 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

species sometimes contain ciguatera toxin. The only tests for its presence use living animals. The most useful bioassays involve feeding flesh or organs of a suspect fish to a mongoose (2) or cat (3) or the intraperitoneal injection of suitable extracts into mice (2); death is the primary result looked for. Research on ciguatera toxins has been hindered by the lack of a sensitive bioassay that consumes little material.

We wish to report a new and sensitive bioassay procedure, utilizing brine shrimp (*Artemia salina* L.) larvae. This procedure was developed with the aid of confirmed ciguatera fish from the Caribbean.

Brine shrimp have been used for the bioassay of fungal toxins (4, 5), dinoflagellate toxins (6), morphine-like analgesics (7), anesthetics (8), and insecticides (9–11). Brine shrimp were unaffected by finely ground ciguatera fish (2). However, we found brine shrimp to be very sensitive to certain organic extracts of toxic fish. It is postulated, therefore, that the ciguatera toxins must be released from storage sites in the flesh and that certain organic solvents can release the toxins.

Rayner (12) demonstrated that at least one ciguatera toxin caused a marked increase in the passive transport of sodium ions across certain cell membranes. We reasoned that an animal, such as *A. salina*, which has a well-developed sodium pump, might be highly sensitive to such a toxin. This prompted an investigation of this animal as a possible test organism.

Six ciguatera fish[1], confirmed to contain the toxin by the mongoose assay or recovery from cases of human poisoning, were obtained. These fish included an amberjack (*Seriola dumerili*), an almico jack (*Seriola rivoliana*), a horse eye jack (*Caranx latus*), a misty grouper (*Epinephelus mystacinus*), a snapper, and an unidentified fish. The specimens were shipped, packed in dry ice, to our laboratory. These specimens were homogenized with acetone in a blender[2], cooled to −20°, filtered, and concentrated (3). The concentrates were extracted with hexane and then ether (13). The highest concentrations of toxin were in the ether extracts; the next highest concentrations were in the *n*-hexane extracts.

Twelve extracts (six ether and six hexane) were ranked one to 12 in potency by comparing the effects of administering 1% polysorbate 60 emulsions intraperitoneally to mice (14). The dose required in the mouse assays on these crude extracts was 30 ml/mouse. Precisely the same ranking of potency was obtained with the brine shrimp. Similar extracts of several specimens of marine fish, shown by the mouse assay (14) not to contain ciguatera toxin, were nontoxic to the brine shrimp. The nontoxic marine fish thus far studied include specimens of amberjack, horse eye jack, spanish mackerel (*Scomberomorus maculatus*), and blue tang (*Acanthurus coeruleus*).

The brine shrimp assay was carried out as follows. Approximately 100 freshly hatched larvae in 1.5 ml of artificial sea water were placed in each well of a spot plate. Then 0.5 ml of a 1% polysorbate 60 suspension of an extract of known concentration was added. Brine shrimp were observed frequently for 20 min and then at 1, 2, 8, and 24 hr. Many shrimp exhibited erratic, nonproductive, swimming movements prior to death. The number of dead shrimp was noted at each reading. The percent of dead shrimp ranged from 50 to 100% in 24 hr with toxic extracts but was less than 5% with nontoxic extracts. Concentrations of crude toxin used varied from 25 to 10 μg/ml. This assay was sufficiently sensitive to assay spots from a TLC plate.

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