Liquid Chromatography of Anticonvulsants in an Inappropriately Drawn (Lipemic) Serum Sample

To the Editor:

Inevitably, blood samples for drug analysis will occasionally be drawn from an inappropriate site. Here we illustrate how such samples can be handled.

A 21-year-old woman was admitted to the hospital for surgical repair of tongue and facial lacerations, multiple fractures of the mandible, a right pneumothorax, and left lateral malleolar fracture, all incurred in an auto accident. Three months earlier, the patient had undergone a craniotomy for removal of posterior fossa glioma, and she was taking phenytoin and phenobarbital at the time of the accident.

While she was in hospital, phenytoin, 100 mg twice daily, and phenobarbital, 30 mg twice daily, were continued. Three days after surgery, Intralipid, a commercially available intravenous fat emulsion, was started for nutritional support at a rate of 40 mL/hour. The next day, blood sample was drawn for determination of phenytoin and phenobarbital “trough” concentrations. When the sample was received at the laboratory it was noted that the serum appeared “milky,” closely resembling serum from hyperlipidemic patients. Investigation revealed that the nurse had drawn the blood from a site just above the Intralipid intravenous site.

We routinely use a modification of the HPLC procedure of Kabra et al. (1) for assay of phenytoin and phenobarbital in serum. The modification consists of adding one-fifth volume of methanol to the mobile phase, and doing the chromatography at ambient temperature rather than the usual 50 °C. The sample is usually prepared by deproteinizing with two volumes of acetonitrile containing the internal standard, and centrifuging. A sample of the supernate is then injected.

Because in this case the sample was grossly lipemic, the following extraction procedure was devised to “clean up” the sample.

Add 1 mL of chloroform/isopropyl alcohol (95/5 by vol) to a mixture of 0.1 mL of serum and 0.2 mL of internal standard solution (5-allyl-5-phenylbarbituric acid, 25 mg/L, in acetonitrile), vortex-mix for 10 s, then centrifuge for 10 min. Transfer the supernate to a clean test tube and evaporate the organic solvent under nitrogen at ambient temperature. Reconstitute the residue with 0.1 mL of mobile phase and inject 10 μL into the chromatograph. Extract a serum control containing phenytoin, 20 mg/L, and phenobarbital, 35 mg/L, at the same time as the lipemic sample.

To confirm that the anticonvulsant assay values by the new extraction procedure were similar to the values obtained on using only deproteinization, we obtained another (this time appropriately-drawn) blood sample exactly 24 h after the lipemic sample. Again, we assayed a serum control at the same time, after protein precipitation.

The results of the anticonvulsant analysis (Table 1) demonstrate that the concentrations of phenytoin and phenobarbital in the patient's serum and the control serum, measured after extraction, were nearly identical to the concentrations measured after deproteinization. The differences in results between the extracted samples and the deproteinized samples did not exceed the quality control limits of the assay and were negligible.

The extraction procedure described here is a useful alternative to deproteinization and direct injection, at least for sera phenytoin and phenobarbital analysis by liquid chromatography. Without an extraction step, the lipemic sample produced interfering peaks on the chromatogram which resulted in a two- to threefold overestimation of the actual concentrations of phenobarbital and phenytoin.

Reference


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