Primidone Analyses: Correlation of Gas-Chromatographic Assay with Enzyme Immunoassay

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Serum specimens from patients on primidone therapy were assayed by two currently available procedures: a commercially available enzyme immunoassay (EMIT) and a published gas-chromatographic procedure. Both procedures were done with commercially available materials and equipment. Results by the two procedures agreed well, which suggests that the two methods could be used interchangeably. For the 94 specimens studied, the correlation coefficient was 0.98, and the least-squares values of slope and intercept were, respectively, 0.97 and 0.51 mg/liter.

Use of primidone (primac lone; 5-ethyl-dihydro-5-phenyl-4,6(1H,5H)-pyrimidinedione) is well established in therapy to control seizures, especially where patients do not respond well to the common barbiturate or hydantoin therapy (1). Intercurrent variation in metabolism of primidone has not been extensively documented (2), but is of interest in monitoring therapy with primidone in specific patients.

A test system for the enzyme immunoassay of primidone has recently become available from Syva Co., Palo Alto, Calif. 94304, as one of their EMIT-AED tests. It offers a rapid means of drug assay appropriate to the emergency treatment situation. Primidone is assayed by gas chromatography in many laboratories. Comparison of results by these two procedures is therefore considered important, especially to laboratories intending to institute either or both of these methods. This work also supplements comparison studies reported for other analysis procedures for anticonvulsant drugs (3, 4).

An often-encountered limitation of the gas-chromatographic procedure results from the insolubility of primidone. The extraction/purification procedure selected for this study gives reproducible analytical recovery of primidone, and the chromatographic pattern has a low background, relatively free of spurious peaks.

Materials and Methods

The samples we used were 94 serum samples from patients, submitted for assay of primidone during a three-month period.

The immunoassay procedure we used to measure primidone was that presented in the package insert supplied with the EMIT primidone assay system (Syva Co.) as described for the Model 24/25 spectrophotometer with sipper system (Beckman Instruments, Inc.). The Model 24/25 was used with the Model 701 printer/calculator (Beckman Instruments, Inc.). Samples were dispensed and diluted with a Model 1800 pipettor/diluter (Cavro Scientific Instruments, Los Altos, Calif. 94022). All components for analysis, including standards and controls,

were part of the assay system supplied by Syva Co., for evaluation of the procedure for primidone analysis. The antibody to primidone that they supplied was described as showing a cross reactivity, relative to primidone as 100%, of 1.25% for phenobarbital, 0.25% for mephobarbital, 0.28% for secobarbital, 1.25% for diphenylhydantoin, 0.25% for methoxuximide, 0.25% for ethosuximide, and 1.25% for carbamazepine.

The gas-chromatographic analysis was done as described by Perchalaki et al. (5), with only minor changes. Trimethylanilinium hydroxide in methanol 25/75 by vol. (Southwestern Analytical Chemicals, Inc., Austin, Tex. 78787) was used for methylation. Primidone for preparation of standards and serum pools was obtained from Ayerst Laboratories (685 3rd Ave., New York, N.Y. 10017). Methylene chloride "spectrograde" (J. T. Baker Chemical Co., Hayward, Calif. 94544) was substituted for ethyl ether as the extraction solvent. 5-Methyl-5-phenylhydantoin (Aldrich Chemical Co., Inc., Milwaukee, Wis. 53233) was used as internal standard, and micro nipple-tipped centrifuge tubes "Concentratubes" (Laboratory Research Company, Los Angeles, Calif. 90036) were used for final extraction. Control samples were prepared by pooling drug-free serum and introducing ethanolic primidone standard in the pool to a concentration of 20 mg/liter. The pooled serum control was stored at -40 °C in 1-ml volume until used. A Hewlett-Packard Model 5710A gas-liquid chromatograph (Avondale, Pa. 19311) was used for this assay with 3% OV17 on 100-120 mesh Gas Chrom Q (Applied Science Labs., Inc., State College, Pa. 16801) as the column packing in a 1.82 m X 2 mm (i.d.) column.

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Results and Discussion

Figure 1 compares results by the two methods. They agree well. Least-squares regression analysis of the 94 specimens showed the slope of the line to be .974, which indicates a proportional error of 2.6%. From the value of the intercept, constant error is estimated at 0.5 mg/liter. Random error is estimated at 1.2 mg/liter from the standard error. The correlation coefficient is 0.976. Thus, for a chromatographic value of 10 mg/liter, the EMIT procedure would give an average value of 10.3 ± 1.2 mg/liter.

Figure 2 shows a gas-chromatographic elution pattern of a patient specimen. As suggested by the pattern, the phenobarbital in the specimen (summation of the three peaks representing phenobarbital) considerably exceeds the primidone concentration. Clinical data are not available to substantiate that all of the phenobarbital is derived from primidone, but this is the sort of pattern usually seen with serum from a patient being treated with primidone.

We find this chromatographic procedure to be highly reliable. It avoids problems encountered with other procedures relative to interfering plasma constituents and solubility of primidone (especially re-solution of the residue after evaporating the initial organic extract). Because both primidone and phenobarbital are quantitated simultaneously, the physician can see the relative amounts of primidone and phenobarbital in the specimen. Day-to-day reproducibility of the chromatographic procedure was determined by 25 replicate analyses of a serum containing primidone, 20 mg/liter. The average concentration was 21.0 mg/liter with a SD of 1.60 and a CV of 7.6%.

Because no concentration or extraction is required, the enzyme immunoassay can be done in 15 min from receipt of the specimen. In fact, the great sensitivity of the system requires that the specimen be diluted as part of the procedure. Day-to-day reproducibility of the EMIT system was determined by 20 replicate analyses of a serum containing primidone, 12 mg/liter. The average concentration was 12.2 mg/liter with a SD of 1.06 and a CV of 8.1%.

References