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Note

Determination of dantrolene sodium in human plasma using high-performance liquid chromatography

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Dantrolene sodium [1-(5-*p*-nitrophenylfurfurylideneamino)hydantoin, sodium salt hydrate] is a muscle relaxant reported to act by blocking muscle contraction beyond the neuromuscular junction. It is used for the symptomatic relief of muscular spasm due to conditions such as stroke, multiple sclerosis, spinal-cord injury and cerebral palsy. Several methods have been described for its detection and quantitation in serum; these methods are based on spectrofluorimetry[1] and differential pulse polarography[2]. The method described here involves a simple protein-precipitation technique from 100 μl of plasma and estimation of the drug by high-performance liquid chromatography (HPLC). It is suitable for the analysis of the drug in plasma down to levels of below 0.25 μg/ml.

MATERIALS AND METHODS

Apparatus

A Waters Assoc. (Milford, Mass., U.S.A.) high-performance liquid chromatograph equipped with a Waters 450 variable-wavelength detector operated at 400 nm was used throughout the determination. The column (30 cm × 4 mm) was packed with μBondapak C_{18} (Waters), and samples were introduced by means of a variable-loop injector (Waters, model U6K). The eluent was 50% acetonitrile in 20 m*M* glycine buffer adjusted to pH 3.5 with 1.0 *M* hydrochloric acid, used at a flow-rate of 1.0 ml/min; under these conditions, the elution time of dantrolene sodium was 4.4 min.

Extraction procedure

To 100 μl of plasma in a pointed tube was added 1.0 ml of acetonitrile carefully down the side of the tube; this ensured that two layers were produced (rapid addition of acetonitrile may cause a lumpy precipitate, which can lead to decreased recoveries). The tube was stoppered and shaken vigorously (manually) for 1 min. The mixture was then centrifuged, and 1.0 ml of the supernatant liquid was transferred to a second tube and evaporated to dryness (50°) in a stream of air. The residue was dissolved in 100 μl of the eluent, and 10–20 μl were injected on to the HPLC column. The peak height obtained was compared with those of a series of standards.

RESULTS

Recovery studies

Amounts of dantrolene sodium were added to blank plasma to give a concentration range of between 0.3 and 10 μg/ml; each sample was then examined by the proposed procedure. The mean recovery from ten such samples was 99 $\pm$ 3%.

Specificity

The use of a wavelength of 400 nm ensured that other pharmaceuticals that could be eluted at a rentention time similar to that of dantrolene showed no response (due to their lack of absorbance). In all the samples that we have examined, the HPLC traces have been free from interfering compounds.

DISCUSSION

As the recoveries obtained were consistent and almost theoretical, an internal standard was not incorporated into the assay. During estimation of the drug in patients, it has been found that a peak elutes just before that of dantrolene sodium (Fig. 1), which may be the 5-hydroxy-metabolite[3]. It has been reported that plasma levels of dantrolene varied widely from patient to patient and were related only roughly to dose[3]; other reports indicate an apparently linear dose–response curve[4]. In order to investigate these results, a simple and rapid analytical procedure was needed. The method described satisfied these criteria, allowing the drug to be estimated at levels down to below 0.25 μg/ml.

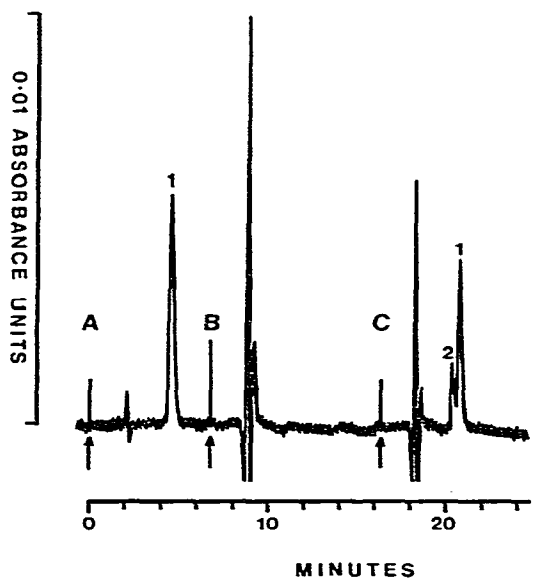

Fig. 1. HPLC analyses. (A) chromatogram of 40 ng of dantrolene sodium; (B) chromatogram of an extract from blank plasma; (C) chromatogram of an extract of plasma from a patient (contains 3.15 μg/ml of dantrolene sodium). Peaks: 1 = dantrolene sodium; 2 = unknown, possibly the 5-hydroxy-metabolite. The arrows mark the time of sample injection, and the vertical bar indicates 0.01 absorbance unit.

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