TITLE: Determination of d-Tubocurarine by Liquid Chromatography

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INTRODUCTION: d-Tubocurarine (dTc) is a monoquaternary ammonium compound used intravenously for muscular relaxation. Despite its widespread use in clinical anesthesia, there is no practical assay for non-research application. This paper describes apparatus, technique and results of our methodology to determine dTc in plasma and urine using liquid chromatography.

APPARATUS: The solvent delivery system was assembled from commercially available parts and is capable of delivering non-pulsatile flows at pressures up to 5000 PSI. Samples are injected through a Valco Loop Valve. The outlet of the column is connected to a Schoeffel UV detector and chromatograms are recorded on a Linear Instrument Strip Chart recorder.

CHROMATOGRAPHIC SYSTEM: The stationary phase is a Waters C18 column at room temperature. The mobile phase consists of 18% Acetonitrile, 82% water, 0.2M perchloric acid at pH 5.4. Flow is 2.5 ml/min.

INTERNAL STANDARD: d-Isochondodendrocurine (ICD) has identical UV absorption characteristics and chemical properties as dTc. It separates easily from dTc on a reverse phase column (Fig. 1).

TECHNIQUE: To one ml plasma we add 1 ml containing 500 mg of the internal standard and 0.1M phosphate buffer at pH 10.5 and 1M of the ion pairing reagent KI. To this, 10 ml of ethylene dichloride is added, the mixture is shaken and then separated. The organic solvent (containing dTc and ICD) is evaporated in a waterbath and the residues are resolvated prior to injection in 50 ul of the mobile phase.

RESULTS: Extraction of dTc is optimal at pH 9.5 and usually yields 75% of the total amount in the sample. The pH of extraction is very critical. Separations on column are best at pH 5.4. The degree of ionization of the mobile phase determines the relative position of the internal standard to the drug of interest. Peak separations (k' > 5) and shape (R > 2000) are excellent for dTc and ICD in water (Fig. 1) and plasma. Although 280 nm is a suitable wavelength for detection in a 10 ul chamber, we found that at 204 nm, absorption was enhanced tenfold. Other compounds also absorb in this range; however, in this system, none was found to be retained on the column longer than dTc or ICD. Quantitation was done manually by calculating peak height ratios. The standard curve thus obtained (Fig. 2) is linear over the whole therapeutic range and accurate to 10 times less than the lowest concentration with therapeutic effect. The relative standard deviation of replicate samples is less than 8%. A single run takes less than 15 minutes, and a total of 24 samples can easily be handled by one technician in a single shift.

CONCLUSION: We have developed a new, rapid, accurate and sensitive chemical assay for the determination of dTc in plasma and urine. The technique is inexpensive and allows for the more precise control of drug levels. The equipment used is standard; thus samples can be assayed in a clinical laboratory at low cost.

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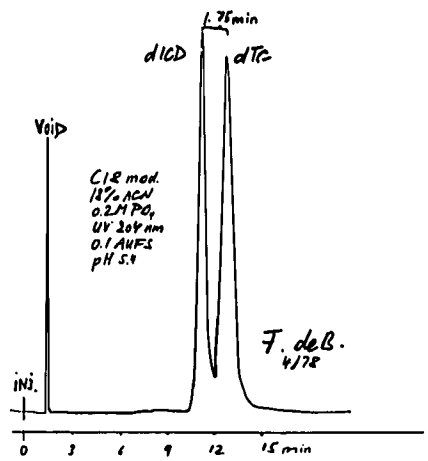

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