Improved Extraction of Aldosterone from Plasma and Urine before Radioimmunoassay

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A rapid extraction method for urinary and plasma aldosterone is discussed in which cellulose extraction columns are used to increase recovery and reduce labor cost.

Radioimmunoassay of urinary or plasma aldosterone requires an initial extraction of steroids before purification and analysis. This extraction characteristically must be performed on acid-hydrolyzed urine or plasma, both of which have been supplemented with tritiated aldosterone, to assess the analytical recovery. After extraction, the organic phase must be washed sequentially with 0.1 mol/liter sodium hydroxide, 0.1 mol/liter acetic acid, and distilled water (1, 2), a laborious process that takes several hours. We sought an alternative, and describe here an improved extraction method for aldosterone that could be applied to other steroids.

Methods and Materials

We used solid-phase extraction tubes (JETUBES), which were originally developed at the Jet Propulsion Laboratory for use in the National Aeronautics and Space Administration program. The JETUBE is simply a polypropylene tube filled with an inert cellulose gauze matrix that has been purified by extensive solvent treatment. The hydrophilic nature of the solid phase allows a liquid–liquid extraction separation of aqueous polar compounds from hydrophobic steroids with selected nonpolar solvents. The large surface area available allows adsorption with a large range of volumes and concentrations. There is no need for agitation or centrifugation; particulates are retained on the column bed. Immediately before use in aldosterone extraction, the tubes are pre-wetted with 5 ml of glass-distilled methylene chloride.

Urinary aldosterone must be hydrolyzed at pH 1 overnight to free aldosterone from the glucuronide. An aliquot (usually 1 ml) is supplemented with tritiated aldosterone, vortex-mixed, and allowed to set for 10 min. Then the pH is adjusted to neutrality (7 ± 0.5) and the volume adjusted to 5 ml (±0.5 ml) with distilled water. The diluted urine is added to the methylene chloride pre-wetted column, which is then eluted with three to four 10-ml portions of methylene chloride, with 2 min between additions. After drying and reconstitution, the eluate is ready for chromatography on Sephadex-LH 20.

Plasma aldosterone need not be hydrolyzed. One to five milliliters of serum or plasma (depending on whether it is from a peripheral or adrenal source) is supplemented with tritiated aldosterone, vortex-mixed, allowed to set for 10 min, diluted to 5 ml with distilled water, and added to the methylene chloride pre-wetted column. Elute with three to four 10-ml portions of methylene chloride, waiting 2 min between additions. Elution is rapid. In this process, more than 95% of the aldosterone is eluted, and the entire extraction takes about 10 min for either plasma or urine.

Results

Analytical recovery. Recovery of aldosterone from the extraction columns had to exceed 95% before their routine use would be considered. Figures 1 and 2 show the elution pattern and percentage recovery of [3H]aldosterone-supplemented urine and plasma. Ninety-five percent of the labeled aldosterone was extracted from urine by 30–35 ml of methylene chloride. Recovery was similar for plasma after 35–45 ml of the solvent. Thus, recovery is a function of eluent volume and allows calibration of columns to obtain the desired recovery.
To assess extraction efficiencies, I determined recoveries at various steps of each method. In the manual method, the specimen is extracted with methylene chloride, and the extract is then washed with dilute base, acid, and water. After drying and reconstitution, the extracted aldosterone is purified by Sephadex-LH 20 chromatography. In the JETUBE extraction method, there was no need for washing, because a clean separation was achieved. Table 1 summarizes the recovery of aldosterone at each step by both methods. A much higher recovery is achieved initially with the JETUBE, so that the ensuing loss in chromatography is not quite so important. In fact, the higher recovery allows the use of a smaller starting volume of plasma, if necessary, because the concentration of unlabeled antigen in the radioimmunoassay reaction is now increased.

Ease of handling. A typical run of 25–30 aldosterone specimens takes more than 2 h to extract manually and wash. The same size run takes less than 30 min with use of JETUBES, and recoveries are better. In addition, problems of emulsion and tube transfers are eliminated.

Interference of contaminants with radioimmunoassay. Because elution of the extraction tube with methylene chloride could potentially add contaminants (as plasticizers or nonpolar residues) from the fibrous JETUBE matrix, we checked for contaminants. An evaporated eluate from a blank tube showed no visible residue, nor did splitting specimens and extracting by both methods show any effect on binding or results. Therefore, I think there is no interference with the assay.

Cost. The labor savings pays the cost of the tubes. The improved recoveries, however, weighed more heavily in our decision to convert entirely to JETUBES.

I conclude that column extraction of aldosterone from plasma and urine results in improved recovery of the steroid, greater accuracy, improved sensitivity, and allows the use of a smaller initial plasma volume, if necessary. The use of these extraction tubes is being investigated for other steroid and drug uses.

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References