Use of Alumina Columns to Prepare Plasma Samples for Liquid-Chromatographic Determination of Catecholamines

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We describe an improved method of sample preparation for liquid chromatographic determination of plasma catecholamines. The catecholamines are extracted from plasma by using small, cheaply-made columns of alumina, with or without prior clean-up on commercially available ion-exchange columns. Advantages of this technique over the conventional batch-extraction method of using alumina are speed, convenience, and improved sample clean-up. In particular, the one-stage method we describe allows results to be reported within 20 min of receiving the sample.

Although determination of plasma catecholamines by "high-performance" liquid chromatography is now routine in many laboratories, sample preparation still presents difficulties. Most commonly, the catecholamines are concentrated and partly purified by adsorption to alumina and elution in a small volume of acid. Because this is essentially a "batch" procedure, it is fairly time-consuming and not readily adapted to automation. Moreover, the resulting clean-up is not always satisfactory, and adequate resolution of the catecholamines from other electro-active compounds in plasma places great demands on the chromatographic system. This has led us (1) and other groups (2, 3) to advocate yet lengthier two-stage procedures for sample preparation.

We have now overcome some of these disadvantages by using small alumina columns (produced quickly and cheaply in the laboratory) for sample preparation. In our routine, two-stage method we perform an initial clean-up, as before (1), on a commercially available cation-exchange column Bond Elut (100 mg of SCX; Analytichem International, Harbor City, CA 90710) and put the buffered eluate through the column of alumina. This saves time and cost as compared with our previous method (1) in which adsorption to the alumina was carried out as a batch technique. We have also used the alumina columns in a rapid, one-step clean-up procedure, described below.

Methods

Preparation of alumina columns. The alumina columns are prepared in plastic pipette tips (Capillettor 50–250 μL; Clinicon International GmbH; Boehringer Corp. (London) Ltd., Lewes, East Sussex BN7 1LG, U.K.). The distal end of this tip consists of a parallel-sided cylinder (3 mm i.d. × 38 mm length), ending in a conical point with a small outlet hole. The wider upper part of the tip is removed, and the conical end is plugged with a disc punched from porous polyethylene (Bond Elut frit material, Analytichem International). A 5-mL pipette tip reservoir is attached to the open end with a short connecting piece of flexible tubing. We use commercially available acid-washed alumina (Bioanalytical Systems Inc., W. Lafayette, IN 47906), which is activated by heating it to 200 °C for 2 h. It then is stored in a stoppered tube and used within one week. Into each of a batch of columns mounted on a vacuum apparatus [pipette washer (no. 1801; Clay Adams, Parsippany, NJ 07054), held inverted in a clamp-stand and connected to a filter pump] we tip 100-mg portions of alumina; 100 mg forms a column 14 mm deep. After use, the columns are emptied, washed, and dried (complete with frit) before re-use.

Two-stage sample preparation. For our routine two-stage method, we mount the 100-mg SCX Bond Elut columns in the vacuum apparatus and prepare them by drawing through 1 mL of methanol followed by 1 mL of phosphate buffer (NaH₂PO₄/N₄HPO₄, 200 mmol/L, pH 6.5). We then draw through 1.5-mL plasma samples containing 40 pmol of 3,4-dihydroxybenzylamine as internal standard, and wash the columns with 10 mL of de-ionized water, discarding all eluates. We then elute the catecholamines with 1 mL of 1 mol/L HClO₄ by centrifugation into tubes containing 100 μL each of 250 mmol/L solutions of EDTA and sodium metabisulfite. To this eluate we add 2 mL of Tris buffer (2 mol/L, pH 8.6) and tip the solution onto an alumina column, drawing it through under gentle suction. (We find a minimum time of 4 min is needed for satisfactory adsorption.) The columns are washed with 15 mL of de-ionized water, and the catecholamines are eluted in 150 μL of 600 mmol/L HClO₄. (We centrifuge the eluate directly into auto-sampler vials for injection into the chromatograph.)

Rapid, one-stage sample preparation. For the single-stage method, 100-mg alumina columns are prepared as above. To 1.5 mL of plasma are added 50 μL each of 250 mmol/L solutions of EDTA and sodium metabisulfite, 1.5 mL of the Tris buffer, and 40 pmol of the 3,4-dihydroxybenzylamine internal standard. Any fibrin or other precipitate is removed by centrifugation (otherwise, it tends to clog the column). The samples are drawn through the columns over at least a 4-min period (see above). The columns are then washed sequentially with 2.5 mL of de-ionized water, 5 mL of 200 mmol/L Na₂CO₃ solution containing EDTA and sodium metabisulfite (5 mmol/L each), and 20 mL of de-ionized water, and the catecholamines are eluted with 150 μL of 600

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mmol/L HClO₄ by centrifugation, for injection into the chromatograph. The inclusion of an alkaline wash step improves the clean-up, particularly by removing uric acid.

Results and Discussion

Two-stage sample-preparation method. Using the routine, two-stage sample-preparation method described above, the chromatographic traces produced (Figure 1A) are of similar quality to those produced by our previous method (I). Analytical recovery of internal standard in 134 plasma samples prepared with this two-stage method has been 59.2 (SD 9.0) %. The percentage recovery of mixed standards (500 pmol of each amine) extracted by this procedure was: norepinephrine 73.6 (SD 12.0), epinephrine 71.0 (SD 9.7), 3,4-dihydroxybenzylamine 72.8 (SD 9.6), and dopamine 74.3 (SD 12.0) (n = 26).

Single-stage sample preparation. The single-stage method, involving only the alumina columns, is faster than a conventional batch-extraction technique with alumina, and produces chromatograms of superior quality (Figure 1B; cf. Figure 1A in ref. I). Some samples still contain interfering peaks, so we prolong the elution (by using less methanol in the mobile phase) to improve separation. We compared results obtained with this one-stage method with those obtained by our former two-stage method (I) (Figure 2). The agreement was good, especially for high concentrations. Analytical recovery of internal standard in 65 plasma samples was 69.0 (SD 12.8) %. Percentage recovery of mixed standards (500 pmol of each amine) extracted by this procedure was: norepinephrine 65.0 (SD 9.9), epinephrine 61.5 (SD 8.0), 3,4-dihydroxybenzylamine 70.6 (SD 5.7), dopamine 69.2 (SD 11.1) (n = 9). Because of the rapidity with which the adsorption and washing procedures can be carried out on the alumina columns, this one-stage method allows results to be reported within about 20 min of receiving the sample. It may therefore be useful in real-time, "beside" assay of plasma catecholamines, which has been advocated in the care of the critically ill (4, 5).

References