Communications to the Editor

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LIQUID CHROMATOGRAPHIC ASSAY OF CLAVULANIC ACID USING A HOLLOW-FIBER POSTCOLUMN REACTOR

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A high-performance liquid chromatographic method has been developed to assay clavulanic acid in serum and urine. Clavulanic acid was separated from background components of serum and urine by using an eluent containing tetrabutylammonium bromide as an ion-pairing agent and methanol as an organic modifier on a C_{18} column. Subsequently, the eluent was introduced into a sulfonated polyethylene hollow fiber (0.25 mm i.d. x 50 cm) suspended in a 2 M sodium hydroxide solution followed by detection at 272 nm. At a clavulanic acid concentration of 0.5 μg/ml in serum samples, the precision (relative standard deviation) was 0.87% (n=10). The limit of detection was about 100 pg at the signal to noise ratio of 3.

KEYWORDS—— clavulanic acid; HPLC; beta-lactamase inhibitor; postcolumn reaction; hollow fiber postcolumn reactor

The lack of suitable detectors in high-performance liquid chromatography (HPLC) for trace and ultratrace analysis in complex matrices prompted the development of postcolumn derivatization.[1] However, the conventional postcolumn derivatization technique requires one pump or more to deliver the reagent solution, mixing tee and reaction coil. They lead to band-broadening, dilution, and noisy and drifting base lines, which reduce the sensitivity gained through derivatization. Recently, Davis and Peterson[2] reported the application of a hollow fiber to a postcolumn reactor for HPLC. In previous papers,[3,4] we reported that clavulanic acid (which is a β-lactamase inhibitor) is rapidly degraded in alkaline methanolic solution to yield a product having an ultraviolet (UV) absorption maximum at 267 nm, and we applied the above reaction to an HPLC method to determine clavulanic acid in plasma and urine. This communication deals with the HPLC assays of clavulanic acid in serum and urine using a hollow fiber as a postcolumn reactor.

Figures 1 and 2 show the separation of clavulanic acid from the background components of serum and urine. Serum samples were ultrafiltered using an Amicon YMT membrane, and the ultrafiltrate was injected onto a column. Urine samples were diluted 10-fold with distilled water and filtered with a 0.45-μm polyacrylate membrane. The filtrate was introduced onto a column. The HPLC conditions were as follows: column, Develosil ODS-5 (4.6 mm i.d. x 15 cm); eluent, 15 mM tetrabutylammonium bromide (TBAB) - 6 mM sodium dihydrogenphosphate - 6 mM disodium hydrogenphosphate - methanol (1 : 1 : 1 : 1, V/V) for serum samples and 15 mM TBAB - 3 mM sodium dihydrogenphosphate - 3 mM disodium hydrogenphosphate - methanol (1 : 1 : 1 : 1.2, V/V) for urine samples; flow rate, 0.8 ml/min; postcolumn reactor, sulfo-

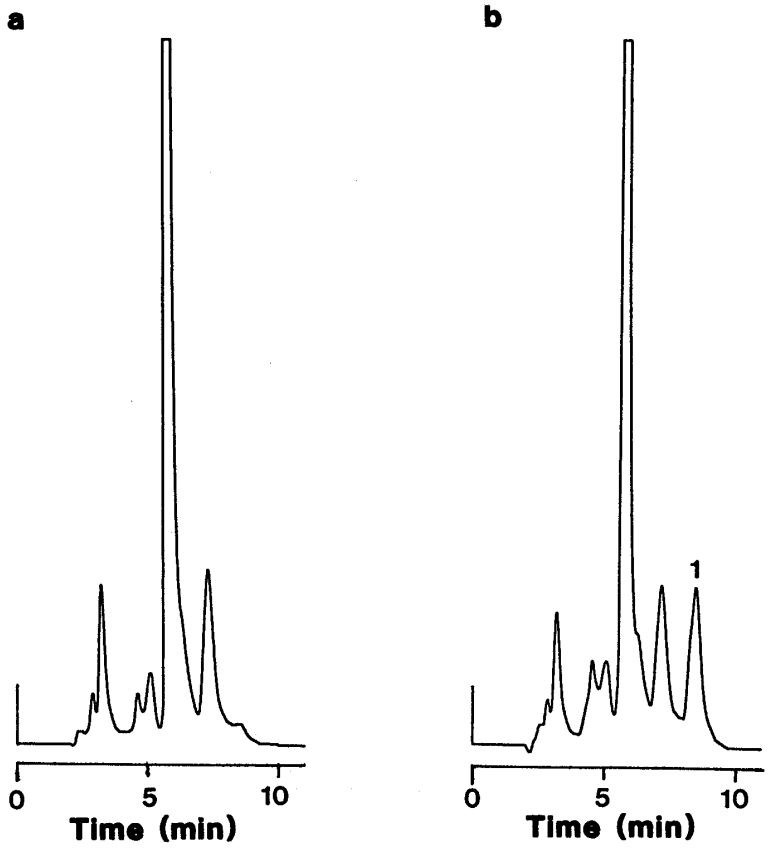

Fig. 1. Chromatograms of Control Serum (a) and Control Serum Spiked with Clavulanic Acid at a Concentration of 0.5 μg/ml (b)

Serum samples were ultrafiltered using an Amicon YMT membrane. Injection volume: 20 μl. Sensitivity: 0.016 aufs. Peak 1 is clavulanic acid.

nated polyethylene hollow fiber (0.25 mm i.d. x 50 cm, AFS-2 fiber, Dionex); detection, 272 nm. All separations and postcolumn reactions were performed at ambient temperature. The hollow fiber reactor was inserted between column and detector and suspended in a 2 M sodium hydroxide solution. The sulfonated polyethylene fiber wall allows sodium ions to permeate inward, so the pH of the eluent is raised to 13. Thus, clavulanic acid is rapidly degraded to methyl 8-hydroxy-6-oxo-4-aza-2-octenoate[3] and detected at 272 nm. The calibration graphs constructed by peak height versus concentration for clavulanic acid were linear in the concentrations ranging from 0.05 to 10 μg/ml for serum samples and from 5 to 100 μg/ml for neat urine samples, and passed through the origin. At a clavulanic acid concentration of 0.5 μg/ml in serum samples, the precision (relative standard deviation) was 0.87% (n=10). The limit of detection was about 100 pg at the signal to noise ratio of 3. The use of the hollow fiber instead of the conventional postcolumn reaction system resulted in a reduction of the base line noise. The proposed method was more sensitive and reproducible than that reported previously.[3,4] These results suggest that the hollow fiber is a good candidate as a postcolumn reactor for HPLC.

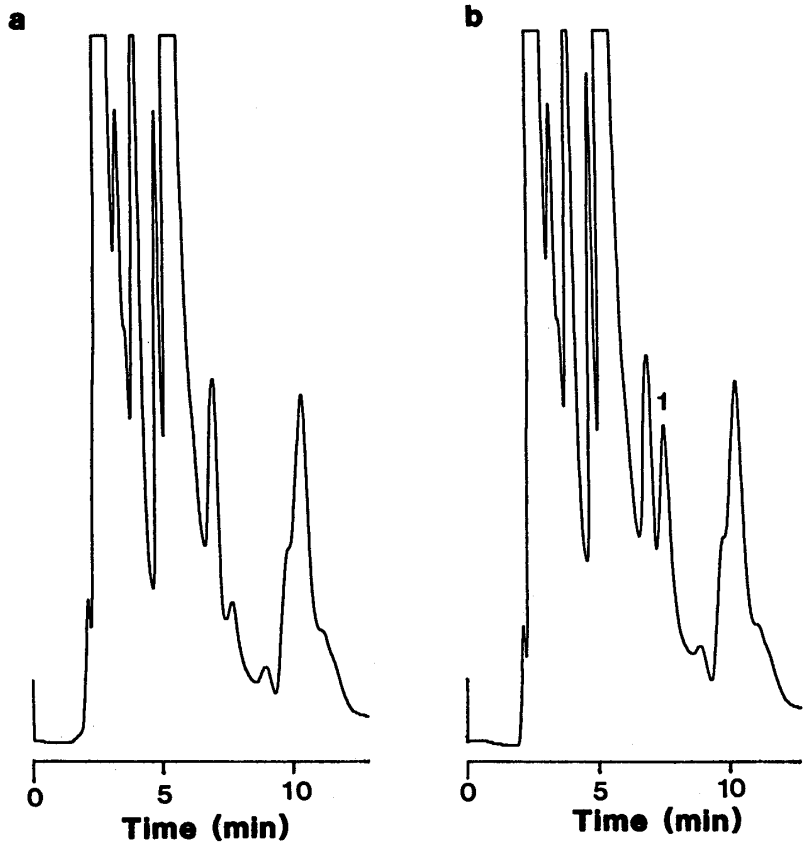

Fig. 2. Chromatograms of Control Urine (a) and Control Urine Spiked with Clavulanic Acid at a Concentration of 5.0 μg/ml (b)

Urine samples were diluted 10-fold with water and filtered with a 0.45-μm membrane filter. Injection volume: 20 μl. Sensitivity: 0.016 aufs. Peak 1 is clavulanic acid.

Previously, we reported the HPLC methods for the determination of sulbactam[4,5] and penicillins[6] using the postcolumn alkaline degradation reaction. We are now investigating the application of the hollow fiber postcolumn reactor to the analysis of sulbactam and penicillins in biological samples.

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