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Note

A simple method for heating coiled columns for high-pressure liquid chromatography

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The liquid chromatographic separation of purine and pyrimidine bases, ribonucleosides and ribonucleotides with long, narrow-bore columns packed with pellicular ion-exchange resins is facilitated by elevated column temperatures[1,2]. In order to use 3-m × 1-mm coiled columns (Reeve Angel Pellionex-SCX and Pellionex-SAX) with a Waters Associates ALC 202 liquid chromatograph, we devised a simple and efficient method for obtaining elevated column temperatures which may be of use to others.

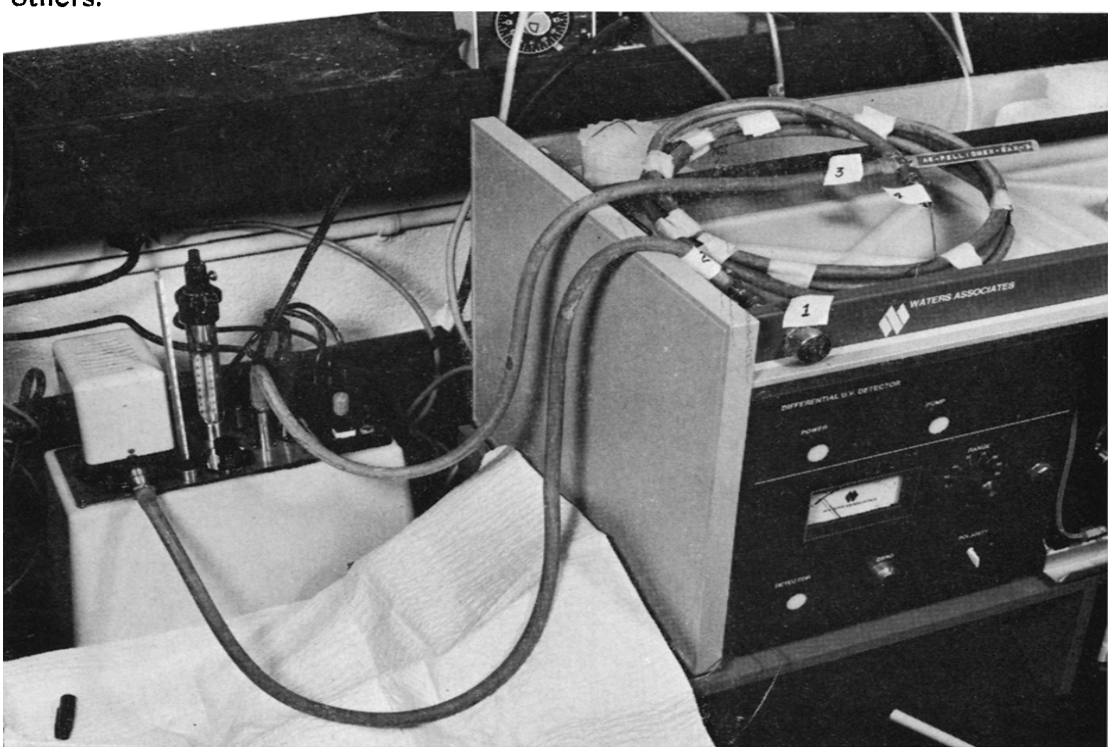

Fig. 1. The Reeve Angel Pellionex-SAX anion-exchange column as it is used with the Waters Associates ALC 202 liquid chromatograph. The numbers in the picture depict: (1) injection port, (2) water inlet, (3) water return, and (4) detector end of column.

Essentially, the column is placed inside an adequate length (~ 10 ft.) of latex tubing (1/4 in. I.D., 7/16 in. O.D.) and water is circulated at the appropriate temperature. We use a Haake, Model FE, circulator bath for this purpose. Each end of the column is fitted through a 1/4-in. Swagelok union tee. Leakage of water around the column is prevented on the jacketed side by forcing the rubber tubing over the tee. A rubber septum (Waters Associates, WRS No. 27288) is cut with a cork borer to fit inside the tee on the opposite side, and a 1/4-in. hexagonal nut is used to hold the septum seal in place. The septum material is such that the narrow-bore column can be forced to pierce through it. Leakage has not been a problem during about one year of continual use. By inserting a three-necked, round-bottom flask between the column and the return side of the circulating bath, we have established that the temperature drop across the column is less than 1° at 80°.

Since the detector end of the column is sealed at the factory, the tee and fitted

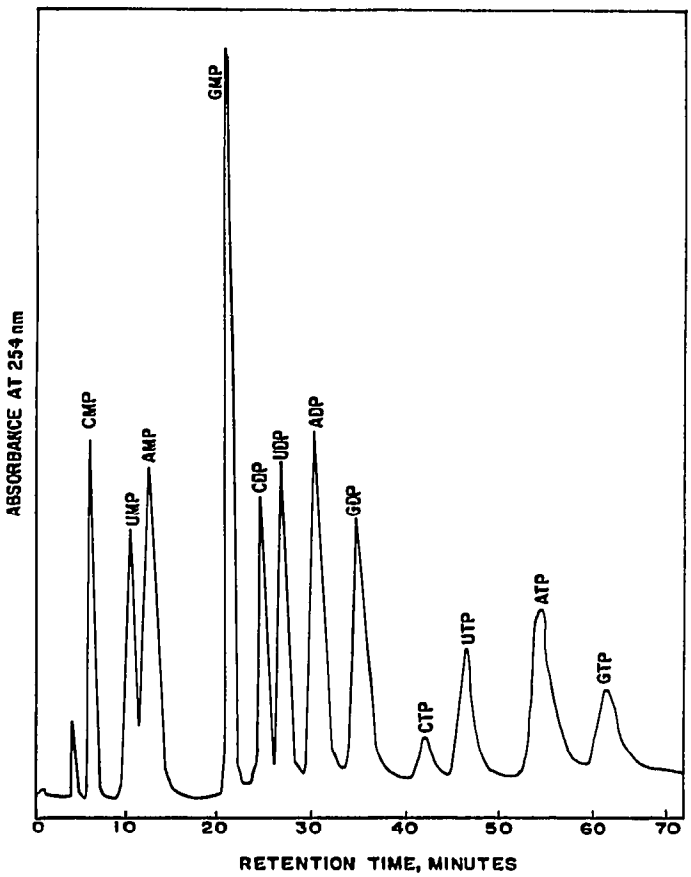

Fig. 2. Representative separation of purine and pyrimidine ribonucleotides. Detection of the eluting compounds was by UV absorption at 254 nm using the micro-flow cell of the ALC 202 instrument and a 10-mV Houston Instruments recorder. Conditions: column, 3-m × 1-mm Pellionex-SAX; temperature, 80°; solvents, (A) 5 m*M* KH_2PO_4, pH 3.35 and (B) 250 m*M* KH_2PO_4–250 m*M* KCl; flow-rate, 0.4 ml/min; gradient, linear (No. 6, Model 660 programmer) (95% A + 5% B)-(30% A + 70% B) in 90 min; ordinate, 0.08 absorption units full scale.

septum seal are inserted from the injector port end of the column prior to threading the column through the rubber tubing jacket. Inserting the column through the rubber tubing is cumbersome when first attempted, and the following method has proven to work satisfactorily in our hands. The 3-m length of column is slowly introduced into the tubing with an undulating motion while standing atop the laboratory bench. Insertion of the column is also easier if the inside of the tubing is wetted with water. The rubber tubing is then forced over each tee and the column is shaped to approximate its original coiled configuration. A picture of a column thus prepared is given in Fig. 1, and a representative separation of ribonucleotides performed by the same column is shown in Fig. 2. Comparing the results shown in Fig. 2 with those of Brown[1] indicates the utility of this manner of heating the column. Furthermore, the circulating water-bath can serve other useful purposes in the laboratory.

ACKNOWLEDGEMENT

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REFERENCES

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2 C. Horvath and S. R. Lipsky, *Anal. Chem.*, 41 (1969) 1227.