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Note

Modification of high-pressure liquid chromatographic nucleotide analysis

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In our laboratory we have been using high-pressure liquid chromatography to analyze free nucleotide pools in biological samples and to investigate alterations in nucleotide profiles caused by disease processes. Using μparticle, chemically bonded strong anion-exchange packings[1], conditions were determined so that mono-, di- and triphosphate nucleotides of adenine, guanine, hypoxanthine, xanthine, uracil, thymine and cytosine could be separated efficiently and reproducibly. The total analysis time, however, was approximately 2 h. Because our studies now require the analysis of a large number of samples, it was important that elution time be reduced, if possible without sacrificing the resolution of the compounds of interest. Therefore, efforts were made to modify operating conditions to obtain comparable profiles in the least time possible.

Using the same instrumentation and column (Partisil 10-SAX; Whatman, Clifton, N.J., U.S.A.) we reinvestigated the operating conditions for the separation of nucleotides. We found that the addition of KCl to the initial eluent reduced the retention times of monophosphate nucleotide peaks. A small change in pH (from 4.5 to

TABLE I

SUMMARY OF PREVIOUSLY REPORTED HPLC CONDITIONS AND MODIFIED CONDITIONS

Parameter	*Original conditions*[1]	*New conditions*
Low concentration eluent	0.007 *F* KH_2PO_4	0.007 *F* KH_2PO_4 0.007 *F* KCl
pH	4.0	4.0
High concentration eluent	0.25 *F* KH_2PO_4 0.25 *F* KCl	0.25 *F* KH_2PO_4 0.50 *F* KCl
pH	4.5	5.0
Flow-rate	1.5 ml/min	2.0 ml/min
Isocratic elution, low concentration eluent	15 min	5 min
Gradient	Linear 0–100% in 45 min	Linear 0–100% in 35 min
Isocratic elution, high concentration eluent	30 min	10 min
Analysis time	90 min	50 min
Reequilibration time	20 min	15 min
Total time required (analysis and reequilibration time)	110 min	65 min

5.0) in the high concentration eluent provided the best means of reducing the retention times of the triphosphate nucleotide peaks. However, for compounds with short retention times, changing the pH value of the low concentration eluent did not improve the separation. It was also found that flow-rate could be increased, times of isocratic elution at both ends of the gradient decreased and slope of the gradient increased without any significant loss in resolution.

The original conditions and our new conditions for separating nucleotides are summarized in Table I and a chromatogram using the original operating conditions shown in Fig. 1 (bottom) and using our new conditions in Fig. 1 (top).

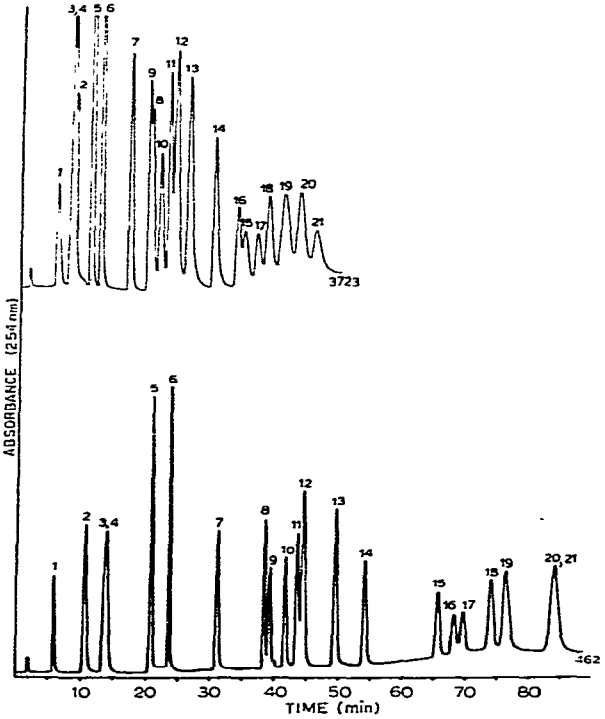

Fig. 1. Comparison of nucleotide analysis. Top chromatogram shows new analysis and bottom chromatogram shows original analysis. 1 = Cytidine-5′-monophosphate; 2 = adenosine-5′-monophosphate; 3 = thymidine-5′-monophosphate; 4 = uridine-5′-monophosphate; 5 = inosine-5′-monophosphate; 6 = guanosine-5′-monophosphate; 7 = xanthosine-5′-monophosphate; 8 = thymidine-5′-diphosphate; 9 = uridine-5′-diphosphate; 10 = cytidine-5′-diphosphate; 11 = inosine-5′-diphosphate; 12 = adenosine-5′-diphosphate; 13 = guanosine-5′-diphosphate; 14 = xanthosine-5′-diphosphate; 15 = uridine-5′-triphosphate; 16 = thymidine-5′-triphosphate; 17 = cytidine-5′-triphosphate; 18 = inosine-5′-triphosphate; 19 = adenosine-5′-triphosphate; 20 = guanosine-5′-triphosphate; 21 = xanthosine-5′-triphosphate.

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