Waters
Lab Highlights
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LITERATURE CORNER

RAPID SEPARATION OF PLATELET NUCLEOTIDES BY REVERSED-PHASE, ISOCRATIC, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH A RADIALLY COMPRESSED COLUMN

Gundu H. R. Rao, Janet D. Peller and James G. White, J. Chromatography, 226 (1981), 466-470.

Human blood platelets contain substantial quantities of adenine nucleotides, as well as smaller amounts of guanine nucleotides. No simple, rapid and efficient assay method for clinical and research samples has been available. The authors describe a method for a complete, isocratic separation of platelet nucleotides on a μBONDAPAK™ C_{18} column. They further present a separation of the same nucleotides in less than 10 minutes using a Radial-PAK™ C_{18} (10μ) with the RCM-100® RCSS at a flow rate of 6 ml/min. The range of recovery was 94-100% of all the nucleotides examined.

"Results of these studies demonstrate that nucleotides can be separated rapidly and quantitated accurately by reversed-phase chromatography using isocratic elution... The method is fast, efficient and provides excellent separation of platelet nucleotides."

See reverse side for chromatograms

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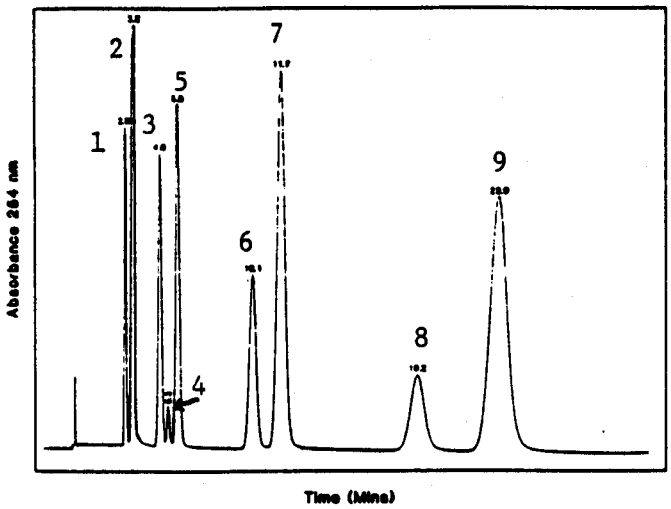
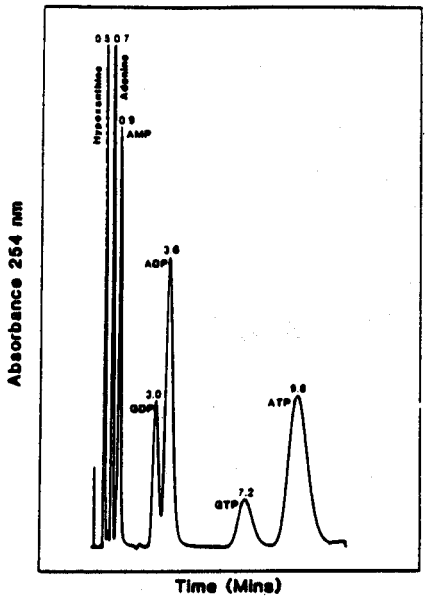


1. Hypoxanthine
2. Adenine
3. c-AMP
4. unknown
5. AMP
6. GDP
7. ADP
8. GTP
9. ATP

Fig. 1. Separation of various nucleotide standards from a mixture by isocratic elution using a μBondapak C_{18} column. Complete separation of all the nucleotides (including c-AMP from AMP) at ambient temperatures was achieved in approximately 24 min. Chromatographic conditions: column, 30 cm × 4 mm; packing, μBondapak C_{18}; solvent, acetonitrile—water—Pic A; detector, UV 254 nm; sample, 10 μl.



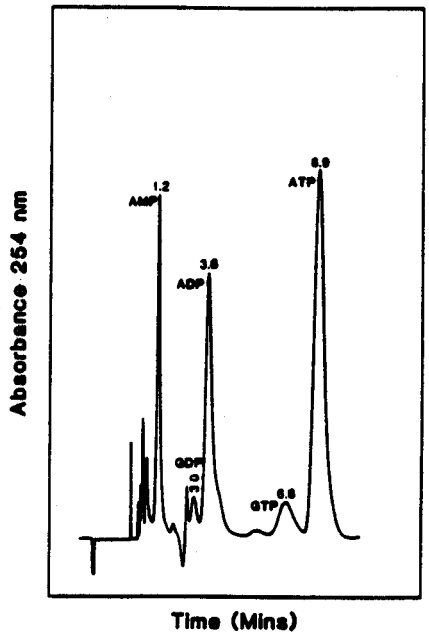


Fig. 2. Separation of various nucleotide standards from a mixture by isocratic elution with a radially compressed column. Elution time for separating all the nucleotides was less than 10 min. The range of recovery for all the nucleotides was excellent. Chromatographic conditions as in Fig. 1 except that column used was Radial-Pak.

Fig. 3. Separation of platelet nucleotides by isocratic elution using a radially compressed column. Identification of individual peaks was done by comparison of retention times of standards added to the platelet extract and assayed under identical conditions. Also, standard additions of known nucleotides to platelet extracts and their analysis served as a supplementary confirmation for peak identification. Retention times for AMP, ADP and ATP were 1.2, 3.6 and 8.9 min. The values obtained using this method for AMP, ADP, ATP and ATP: ADP ratios, agree with results obtained by others using linear gradient elution to achieve complete separation of nucleotides. Chromatographic conditions as in Fig. 2.

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