CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Nucleotide Separations on the BAS 200

A Gradient Elution Method with Time-Programmable Detector Operation.

Introduction

Nucleotides are essential constituents of nucleic acids and enzyme cofactors. They also play an important role in the regulation of cellular functions. Defects in purine or pyrimidine metabolism can be manifested in mental retardation, cardiovascular diseases, renal failure, gout and toxemia.

Liquid chromatography provides a rapid, reliable method for the determination of nucleotides. The method is versatile in that a wide range of nucleotides can be detected using UV absorption detection and gradient elution.

Gradient and detector conditions can be time-programmed during the run to optimize separation and detection parameters, depending on the sample composition and the expected amounts of nucleotides.

Conditions

Instrument: BAS 200 equipped with BAS variable wavelength UV detector. The detector is time-programmable with respect to selection of wavelength, detector gain, and electronic noise filtering.
Wavelength: 254 nm
Column: 25 cm x 4.6 mm 5 Biophase ODS
Mobile Phase A: 0.02 M KH_2PO_4, pH=6.5
Mobile Phase B: 50% Mobile Phase A/50% CH_3CN
Flow Rate: 1.0 mL/min.
Detector Gain: 0.05 AUFS
Linear Gradient: 100%A for 3 min. then to 50% A/50% B at 48 min. Back to 100% A at 68 min.
Amount Injected: 25 µL
Sample: 5' mixed nucleotide standard, ~ 20 µg/mL each

Results and Discussion

The chromatogram depicted in F1 utilized the above conditions for an injection of 25 µL of a mixed nucleotide standard consisting of 5'-mono-, di- and triphosphates. The peaks were tentatively identified by retention as follows:

1) 5'-CMP (cytidinemonophosphate)
2) 5'-UMP (uridinemonophosphate)
3) 5'-GMP (guanidinemonophosphate)
4) CDP (cytidinediphosphate)
5) GDP (guanidineidphosphate)
6) ADP (adeninediphosphate)
7) GTP (guanidinetriphosphate)
8) ATP (adenosinetriphosphate)
9) Cluster of solvent impurity peaks stripped during gradient.

F2 was generated using gradient conditions as shown below:

Time (min)	%A	%B	%C
0	100	0	0
45	50	50	0
50	50	50	0
55	100	0	0
65	100	0	0

Gain changes were also programmed into the detector file as follows:

Time (min)	Gain (AUFS)
0.0	0.05
13.5	0.2
18.0	0.05
20.0	0.2
38.0	0.05
46.0	1.0

Fifty microliters of a mixed nucleotide standard was injected. The standard was a mixture of 5'-mono, di- and triphosphates and 2' and 3' guanidine and

adenine monophosphates. The individual peaks were not unequivocably identified but are presumed to be:

1) 5'-CMP	7) 3'-AMP
2) 5'-UMP	8) 2'-AMP
3) 5'-GMP	9) ADP
4) 3'-GMP	10) GTP
5) GDP	11) CTP
6) 2'-GMP	12) ATP

References

1. B. Allinquant, C. Musenger and E. Schuller, J. Chromatogr., 326(1985) 281-291.

2. A.M. Pimenov, Yu. V. Tikhonov, I.S. Meesner and R.T. Toquzou, J.Chromatogr., 365(1986) 221-227.

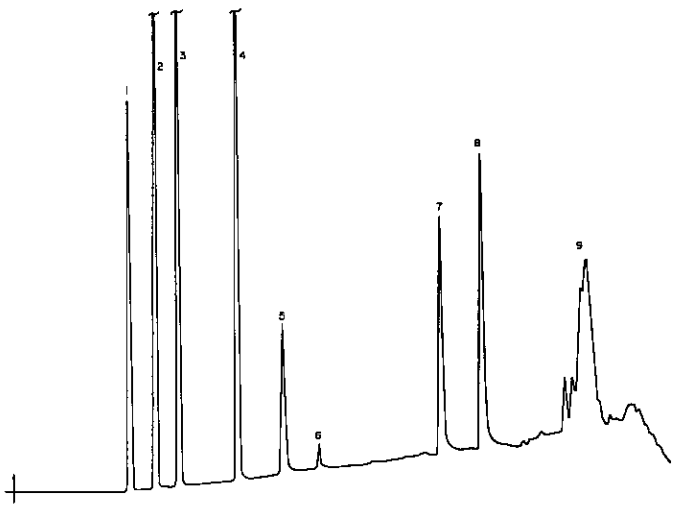

Figure 1. Mixed Nucleotide Standard. Conditions per text.

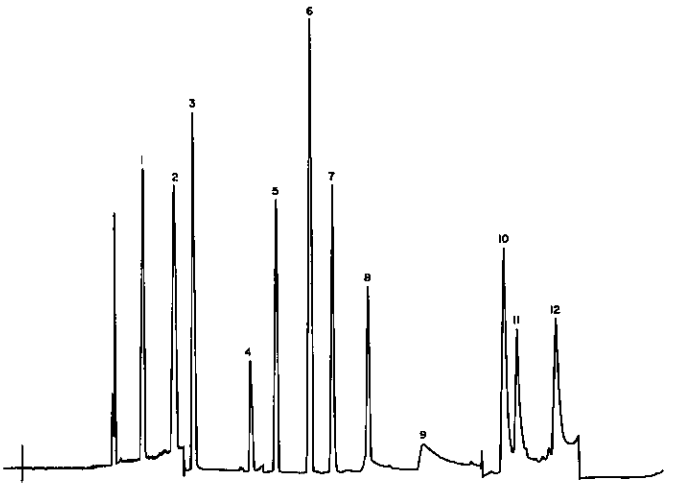

Figure 2. Mixed Nucleotide Standard. Conditions per text.