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This article is archived from a past issue of The Supelco Reporter. Information in the article was appropriate at the time of publication, but product specifications, catalog numbers, and availability may have changed over time.

If you have questions about applying methodology described in this article to a current application, please contact our technical service chemists.



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Rapid, Reliable Nucleotides Analyses, Using Conventional or Ion Pairing Reversed Phase HPLC

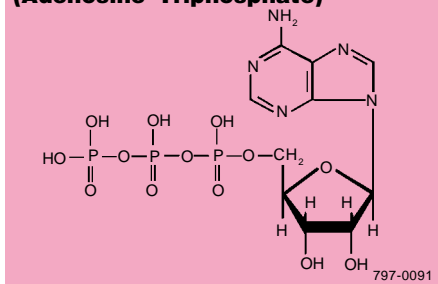
T. Ascah, C. DebRoy, Liquid Separations, Supelco, Bellefonte, PA, USA

SUPELCOSIL LC-18-T columns provide the high efficiency and selectivity needed for analyses of nucleotides and nucleotide metabolites. Special treatment of the silica surface reduces the potential for interactions with these sensitive compounds.

Nucleotides are the basic units of which deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are composed. Intracellular free nucleotides provide chemical energy for many biochemical reactions. Knowledge of the type, concentration, and distribution of nucleotides in a cell gives insight into the cell's metabolic condition. Each nucleotide consists of a purine or pyrimidine base, a pentose sugar (ribose or deoxyribose), and one, two, or three phosphate groups (Figure A).

Reversed phase HPLC (RP-HPLC) on a

Figure A. Typical Nucleotide (Adenosine Triphosphate)



SUPELCOSIL™ LC-18-T column provides the high efficiency and selectivity needed to detect the least common nucleotides, or monitor changes in nucleotide levels during cellular metabolism. RP-HPLC analyses are more stable than traditional ion-exchange analyses, without the mobile phase limitations that plague the latter methods.

SUPELCOSIL LC-18-T columns are designed specifically for efficient, reliable analyses of nucleotides and nucleotide metabolites. Special treatment of the silica surface reduces the potential for silanol or metal ion interactions with these small, charged, very hydrophilic compounds. Both types of undesired interactions cause the analyte peaks to tail, reducing resolution and the sensitivity of the analysis.

Nucleotides can be analyzed on a SUPELCOSIL LC-18-T column through conventional RP-HPLC or ion pairing RP-HPLC. In conventional RP-HPLC separations, nucleotides elute in order of decreasing number of phosphate groups, because the hydrophobicity of the molecule increases. In ion pairing RP-HPLC, the ion pair reagent associates with the phosphate groups, creating a more hydrophobic complex, and increased retention, as the number of phosphate groups increases.

Figure B shows a mixture of nucleotides on a 25cm x 4.6mm SUPELCOSIL LC-18-T column packed with 5µm particles. Analysis time can be significantly reduced, with little sacrifice in resolution, by using a 15cm x 4.6mm column containing 3µm particles. The peak shape is excellent for either column.

Figure B. Nucleotides on a SUPELCOSIL LC-18-T Column

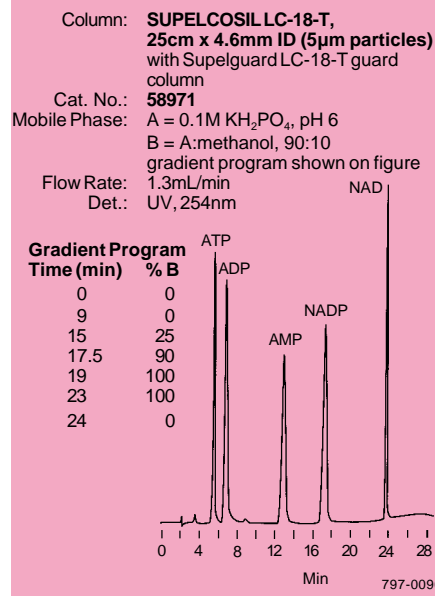


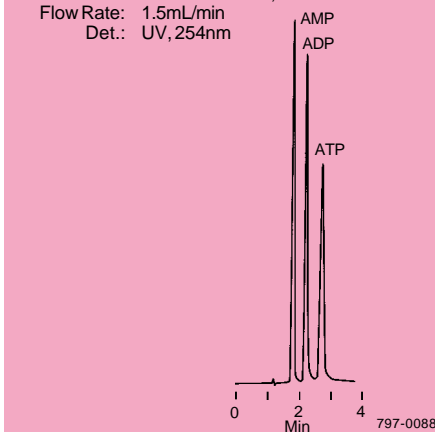
Figure C shows an ion pairing analysis, in which the nucleotides elute in the reverse order of that in Figure B. The analysis time – less than three minutes – shows the value of the 3µm particles for rapid analyses. The 15cm x 4.6mm, 3µm SUPELCOSIL LC-18-T column also is effective for gradient ion pair methods, and will shorten equilibration time.

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Our new Bulletin 913 contains much additional information on nucleotides analyses and shows gradient ion pair analyses on SUPELCOSIL LC-18-T columns. In these analyses the elution pattern can be finely adjusted by making slight adjustments to the mobile phase.

Figure C. Isocratic Ion Pair Separation of Nucleotides

Column: SUPELCOSIL LC-18-T, 15cm x 4.6mm ID (3µm particles) with Supelguard LC-18-T guard column
 Cat. No.: 58970-U
 Mobile Phase: A = 0.1M KH₂PO₄ + 4mM tetrabutylammonium hydrogen sulfate, pH 6
 B = A:methanol, 70:30, pH 7.2 isocratic elution, A:B = 15:85
 Flow Rate: 1.5mL/min
 Det.: UV, 254nm



Ordering Information:

| Description | Cat. No. |
|---|----------|
| SUPELCOSIL LC-18-T Modular HPLC Columns | |
| 15cm long, 3µm particles | |
| 4.6mm ID | 58970-U |
| 4.0mm ID | 58970C40 |
| 3.0mm ID | 58970C30 |
| 25cm long, 5µm particles | |
| 4.6mm ID | 58971 |
| 4.0mm ID | 58971C40 |
| 3.0mm ID | 58971C30 |
| Columns of other dimensions are available as custom products. | |

We strongly recommend using a Supelguard™ LC-18-T guard column to protect the analytical column.

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