Application Note: ANCCSCETNUCLEOT

Analysis of Nucleotides Using Core Enhanced Technology Accucore HPLC Columns

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Key Words

- Accucore aQ
- Solid core
- Superficially porous
- Nucleotides
- ATP
- ADP
- AMP
- CMP
- GDP

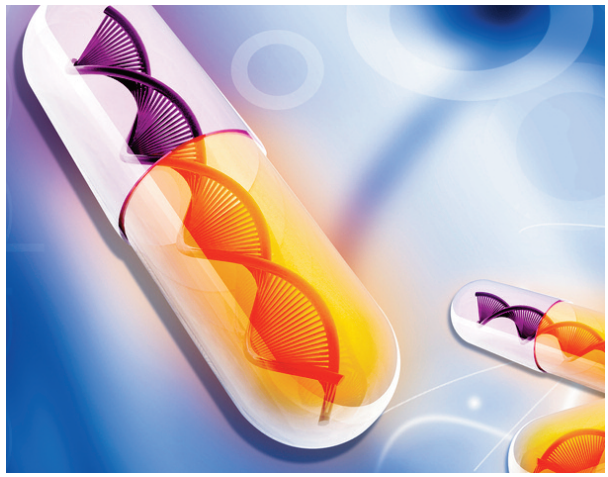
Abstract

This application note demonstrates the fast analysis of nucleotides using a Thermo Scientific Accucore aQ column. The analysis takes less than five minutes and the pressure is suitable for conventional HPLC instruments.

Introduction

Accucore™ HPLC columns use Core Enhanced Technology to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials. The polar functional group in the Accucore aQ, which is a polar endcapped C18 phase, provides a controlled interaction mechanism by which polar compounds can be retained and resolved and enables the use of 100% aqueous mobile phases.

Nucleotides are molecules which can be assembled into DNA or RNA and are a source of energy for biological systems and their analysis is required in a number of different research areas. They are involved in cell signaling, metabolism and are important in many enzymatic reactions. Nucleotides are found in many biological food products such as milk and meat and are believed to have a positive effect on nutrition, especially for infants so they are added to many infant formulae. They are important in drug activity measurements as the activity of an enzyme can be measured by its turnover of a nucleotide, commonly adenosine 5-triphosphate (ATP). Nucleotides occur in blood and body fluids and are important metabolites that are followed in clinical drug trials. This method describes the fast and efficient chromatographic determination of five nucleotides under isocratic HPLC conditions.



Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific water (HPLC grade)	W/0106/17
Sigma-Aldrich Potassium phosphate dibasic ACS reagent	
Sigma-Aldrich ATP, ADP, AMP, GDP, CMP sodium salts	

Sample Handling Equipment	Part Number
Liquid handling hardware:	
Thermo Scientific Finnpipette F2 pipettor kit 10 µL – 100 µL, 100 µL – 1000 µL	PMP-020-220F
Thermo Scientific Finntip pipette tips, 200 µL	PMP-107-600F
Thermo Scientific Finntip pipette tips, 1000 µL	PMP-103-206K
Vials and closures:	
Thermo Scientific borosilicate glass vials (2 mL, 12 mm x 32 mm) with 8 mm black screw cap fitted with a silicone/PTFE seal	60180-600

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific HPLC system	
Column:	Accucore aQ 2.6 µm 100 x 2.1 mm	17326-102130
Measured pressure (average):	137 bar	
Mobile phase:	Aqueous potassium phosphate 50 mM, pH 6	
Flow rate:	0.7 mL/min	
Run time:	5 minutes	
Column temperature:	30 °C	
Injection details:	2.0 µL	
UV detector wavelength:	260 nm	

Solutions

Calibration standard preparation: cytidine 5-monophosphate (CMP), guanosine 5-diphosphate (GDP), adenosine 5-monophosphate (AMP), adenosine 5-diphosphate (ADP) and adenosine 5-triphosphate (ATP) individual primary standards were prepared in water, at concentrations of 5 mg/mL. A mixed working standard was prepared by combining 50 µL of each primary solution and diluting with water to a total volume of 1 mL.

Results

Under the conditions adopted for this analysis retention and separation of six nucleotides can be accomplished in less than five minutes on an Accucore aQ column. The chromatography is presented in Figure 1. In Table 1 the retention times, asymmetry and peak areas are summarized for six replicate injections. The relative standard deviations in the retention times are less than 0.1 % and less than 1.7 % for the peak areas indicating excellent reproducibility over these injections. The retention times and peak areas are highly stable even in an 100 % aqueous mobile phase.

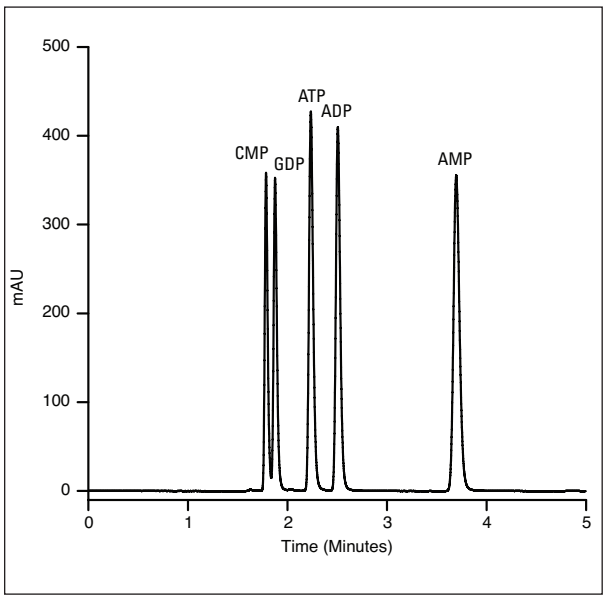


Figure 1: Liquid chromatography of five nucleotides using an Accucore aQ column

Analyte	t_R (minutes)	% RSD t_R	Asymmetry (10 %)	Area	% RSD Area
CMP	1.79	0.05	1.24	742065	1.63
GDP	1.88	0.06	1.21	816602	1.56
ATP	2.24	0.04	1.22	1251785	1.61
ADP	2.51	0.03	1.19	1254007	1.55
AMP	3.70	0.03	1.16	1508682	1.52

Table 1: Data from six injections for average retention time (t_R), percentage relative standard deviation (RSD) in t_R, average asymmetry at 10 % height, average peak area and percentage RSD in the peak area of the five nucleotides analyzed

Conclusions

An Accucore aQ column provides fast separation of a mixture of five nucleotides. The retention times and peak areas are highly stable even in an 100 % aqueous mobile phase.

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