Analysis of phosphate compounds with the Agilent 1260 Infinity Bio-inert Quaternary LC System

Application Note

Biopharmaceuticals

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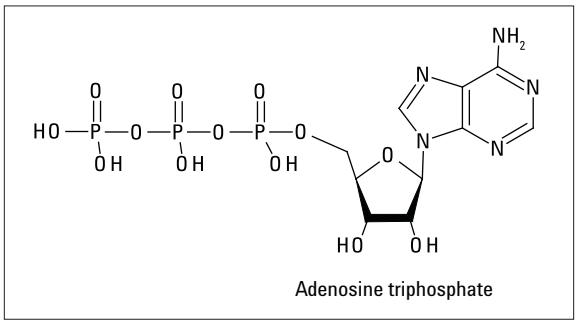

Adenosine triphosphate

Abstract

This Application Note shows that unwanted retention and peak tailing of adenosine triphosphate can be completely prevented when using the Agilent 1260 Infinity Bio-inert Quaternary LC System. Due to the iron and steel-free design of the Agilent 1260 Infinity Bio-inert Quaternary LC System, phosphate compounds can be analyzed without any issues regarding the formation of phosphate-iron complexes found with stainless steel systems. Even in the presence of high organic mobile phases, good peak shapes were observed without peak tailing or area reduction.

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Introduction

Severe peak tailing of phosphate compounds is a well described issue in HPLC analysis[1, 2]. It has been reported for a variety of adenosine and guanidine mono-, di- and triphosphate nucleotides and other phosphate compounds in different HPLC separation techniques[3, 4]. Interaction between stainless steel and phosphate groups were described by Liu *et al.* leading to the formation of phosphopeptide-Fe(III) complexes[5]. With the use of PEEK tubing instead of stainless steel tubing, the peak tailing can be reduced[1].

PEEK has a limited backpressure tolerance, therefore, UHPLC is not possible with PEEK-only capillaries. In addition, standard systems also have stainless steel injector parts and detector flow cells, making them not fully bio-inert. The 1260 Infinity Bio-inert Quaternary LC System has capillaries that consist of PEEK inside and stainless steel outside, enabling UHPLC with a metal-free sample flow path[6]. With this system, a user can analyze a variety of phosphate compounds without the emersion of peak tailing or other unwanted sample retention in the system due to phosphate-iron complexes.

The analysis of adenosine triphosphate (ATP) has been employed as a measure of microbial biomass[7], to determine the energy status in plant tissues[8] and in various other metabolic analytical experiments.

Experimental

The Agilent 1260 Infinity Quaternary LC System used for the experiments consisted of the following modules:

- Agilent 1260 Infinity Quaternary Pump (G1311B)
- Agilent 1260 Infinity Standard Autosampler (G1329B)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1260 Infinity Diode Array Detector SL (G1315C), equipped with standard flow cell, 10 mm

The Agilent 1260 Infinity Bio-inert Quaternary LC System used for the experiments consisted of the following modules:

- Agilent 1260 Infinity Bio-inert Quaternary Pump (G5611A)
- Agilent 1260 Infinity High Performance Bio-inert Autosampler (G5667A)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1260 Infinity Diode Array Detector VL (G1315D), equipped with bio-inert standard flow cell, 10 mm

A PEEK restriction capillary was used instead of a stainless steel column.

Software
Agilent OpenLAB CDS, ChemStation Edition for LC & LC MS Systems, Rev. C.01.02 [14]

Solvents and Samples

Buffer A: 10 mM ammonium acetate

Buffer B: 10 mM ammonium acetate + 10% methanol

Buffer C: 10 mM ammonium acetate + 50% methanol

Buffer D: 10 mM ammonium acetate + 70% methanol

Buffer E: 10 mM ammonium acetate + 90% methanol

Sample
Adenosine triphosphate (ATP), solved in H_2O_{dd} (5 mg/mL)

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). Ammonium acetate and ATP were purchased from Sigma-Aldrich, St.Louis, USA.

Chromatographic conditions

Flow rate:	0.5 mL/min
Isocratic run with buffer A, B, C, D or E	
Stop time:	5 minutes
Injection volume:	0.2 µL
Temperature TCC:	40 °C
Diode array detector:	254 nm, Reference 360 nm
Peak width:	> 0.1 minutes (2.5 Hz)

Results and discussion

Significant peak tailing could be observed for ATP analysis in a stainless steel based system, the 1260 Infinity Quaternary LC System. With increasing amount of organic mobile phase, the retention of the phosphate compound was increasing to a huge extent, also resulting in relevant area reduction. With the use of 90% MeOH in the mobile phase, complete retention of the ATP was observed, see Figures 1, 3, and 4.

With the 1260 Infinity Bio-inert Quaternary LC System, the unwanted retention of the used phosphate sample could be completely prevented, resulting in good peak shapes without substantial peak tailing or area reduction, see Figures 2, 3, and 4.

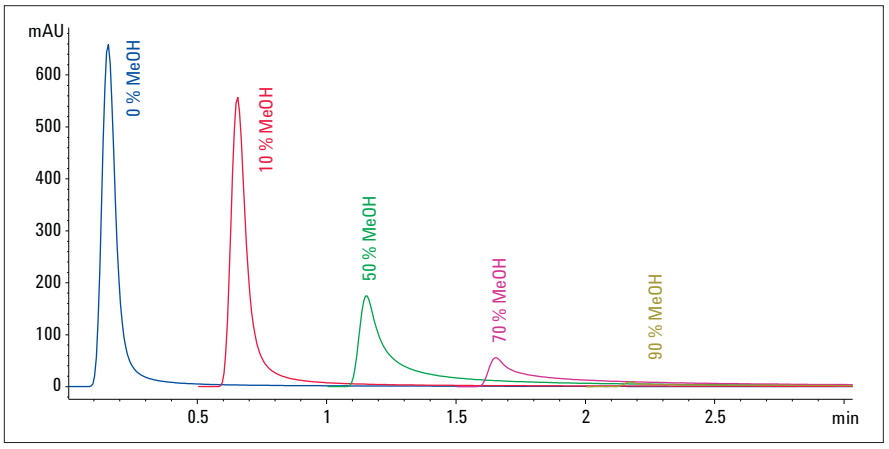

Figure 1
ATP analysis on the Agilent 1260 Infinity Quaternary System (stainless steel system).

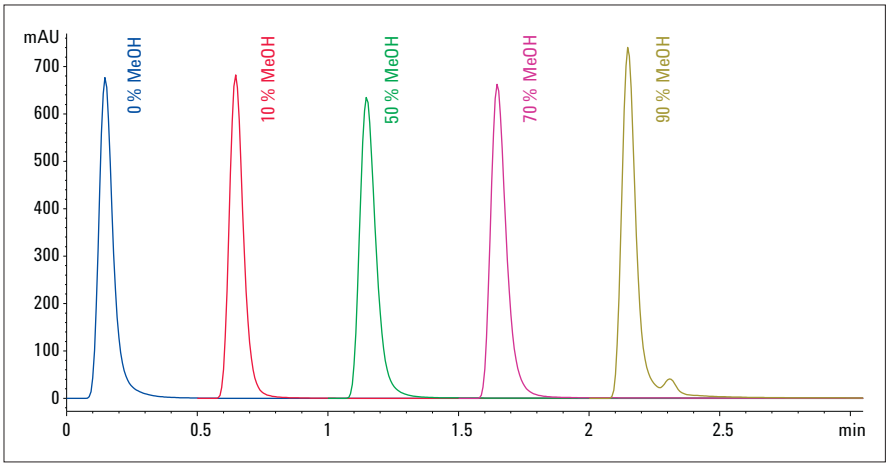

Figure 2
ATP analysis on the Agilent 1260 Infinity Bio-inert Quaternary System.

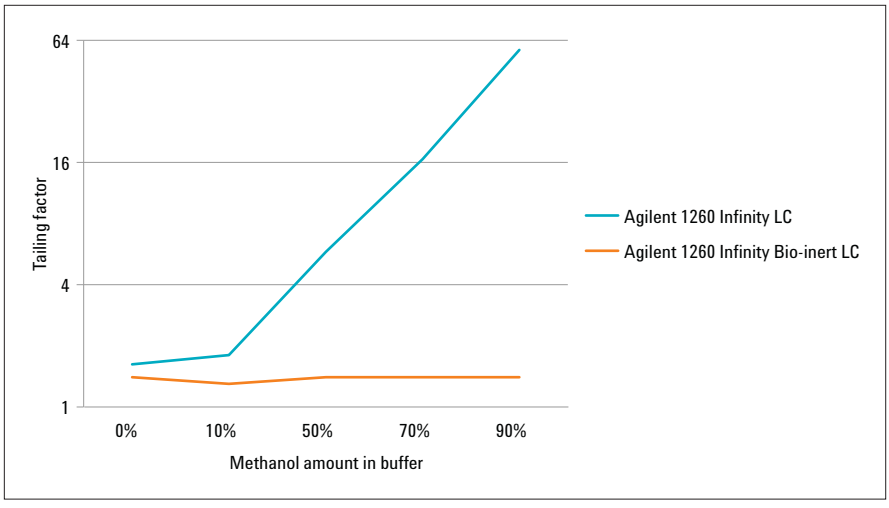

Figure 3
Tailing factors of ATP relating to the MeOH amount in the used buffers.

Conclusions

Unwanted retention and peak tailing of adenosine triphosphate could be completely prevented when using the Agilent 1260 Infinity Bio-inert Quaternary LC System. In contrast to the use of a stainless steel system, good peak shapes were observed. Peaks without substantial peak tailing or area reduction due to extensive sample retention in the flow path were found even with increasing organic content in the used mobile phases.

Due to the iron and steel-free design of the 1260 Infinity Bio-inert Quaternary LC system, phosphate compounds can be analyzed without any issues regarding the formation of phosphate-iron complexes as found with stainless steel systems.

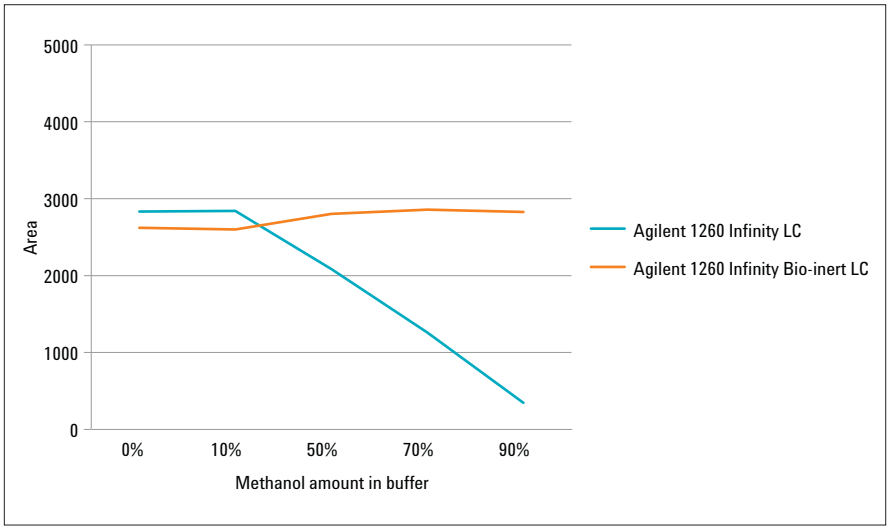

Figure 4
Area of ATP relating to the MeOH amount in the used buffers.

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Published in the USA, March 1, 2012
5991-0025EN

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