Proof of Performance

Analysis of compounds in mobile phases with high pH

Feasibility of the Agilent 1260 Infinity Bio-inert Quaternary LC system for generic high pH applications

Application Note

Chemical and Pharmaceutical Analysis

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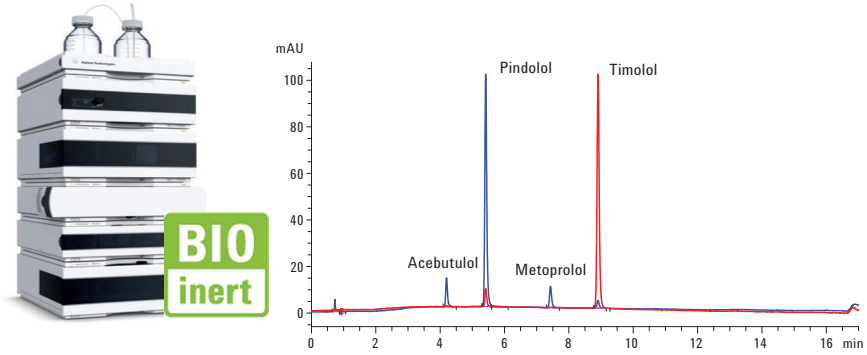

Abstract

In this Application Note, the feasibility of the Agilent 1260 Infinity Bio-inert Quaternary LC system for generic high pH applications is demonstrated. The iron and steel-free design enables the user to work in a wider pH range (1–13, and up to 14 for short periods) compared to the stainless-steel based Agilent 1260 Infinity LC system. Retention time and resolution stability over a time period of 20 hours are demonstrated for the analysis of four ß-blockers, serving as examples for small molecules. A tryptic BSA digest served as an example for biomolecules and showed retention time stability over a time period of 37.5 hours. This confirms that high pH mobile phases (pH 10 to 11) have no adverse effects on the stability of retention time or resolution for the analyzed samples over extended periods.

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Introduction

Performing high pH liquid chromatography in pH ranges above 10 puts stainless-steel systems at high risk of damage after long-term use due to corrosion effects. It is, therefore, highly recommended to use an iron-free system that is not deteriorated by aggressive agents used in high pH applications.

The Agilent 1260 Infinity Bio-inert Quaternary LC consists of metal-free components in the sample flow path. All capillaries and fittings throughout the autosampler, column compartment, and detectors are completely metal-free so that bio-molecules interact only with ceramics or PEEK. This assembly type (iron and steel-free design) enables the user to deploy not only buffers containing high amounts of salt but also to work in a wider pH range (1–13, and up to 14 for short periods) compared to the Agilent 1260 Infinity LC system. Further, it has been already proven that the 1260 Infinity Bio-inert Quaternary LC system releases less metal ions in presence of high pH eluents (for example 100 mM NaOH) compared to the 1260 Infinity LC system[1].

In this Application Note, the feasibility of the 1260 Infinity Bio-inert Quaternary LC system for generic high pH applications is demonstrated. ß-Blockers, as examples for small molecules and a peptide mix (tryptic BSA digest) as an example for bio-molecules were used as samples, analyzed at high pH[2,3]. The influence of high pH eluents on the stability of retention time and resolution was investigated.

Experimental

The Agilent 1260 Infinity Bio-inert Quaternary LC system consisted of the following modules:

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

Column

Agilent ZORBAX Extend-C18, 4.6 × 100 mm, 1.8 µm, 600 bar

Software

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

Eluents and samples

1. 50 mM triethylamine (TEA, **pH 11**) and methanol, for the analysis of four ß-blockers:
   - Acebutulol
   - Pindolol
   - Metoprolol
   - Timolol
2. 15 mM ammonium hydroxide (NH_4OH, **pH 10**) and acetonitrile for peptide analysis

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). TEA, NH_4OH and the ß-blockers were purchased from Sigma-Aldrich, St.Louis, USA.

Chromatographic method

	ß-Blockers	Peptide mix (Tryptic BSA digest)
Solvents:	A: 50 mM TEA, pH 11 B: Methanol	A: 15 mM NH_4OH, pH 10 B: 90% Acetonitrile, 15 mM NH_4OH, pH 10
Gradient:	0 min – 45% B 15 min – 80% B 15.01 min – 95% B	0 min 5% B 25 min – 30% B 30 min – 100%
Stop time:	17 min	35 min
Post time:	3 min	10 min
Temperature:	35 °C	35 °C
Flow rate:	1 mL/min	1 mL/min
Injection volume:	3 µL	3 µL
DAD:	260 nm, reference 360 nm 300 nm, reference 400 nm	214 nm, reference 400 nm
Peak width:	0.025 min (0.5 s response time) (10 Hz)	<0.013 min (0.13 s response time) (20 Hz)

Results and Discussion

High pH Analysis of Small Molecules

Four different ß-blockers were analyzed using TEA as basic aqueous buffer (pH 11) and methanol as organic mobile phase. Figure 1 shows the chromatogram after detection by two different wavelengths. The first three ß-blockers (Acebutulol, Pindolol and Metoprolol) were detected at 260 nm and Timolol at 300 nm. RSD of retention time was < 0.2% for all four peaks.

Retention time stability was monitored over a time period of 20 hours and remained stable from 0 to 1,200 minutes, see Figure 2.

Resolution stability for Pindolol, Metoprolol and Timolol was monitored over a time period of 20 hours and remained stable from 0 to 1,200 minutes, see Figure 3.

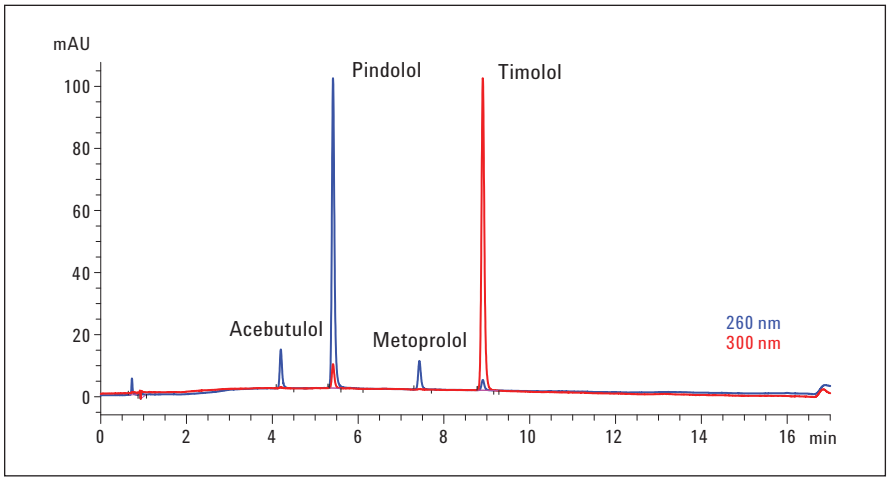

Figure 1
LC chromatogram of the four ß-blockers, analyzed with basic mobile phase at pH 11.

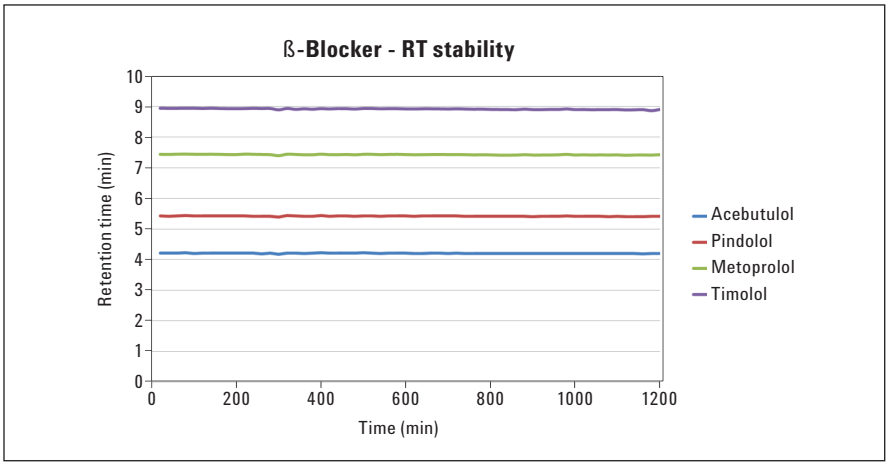

Figure 2
Retention time stability of four ß-blockers, analyzed at high pH.

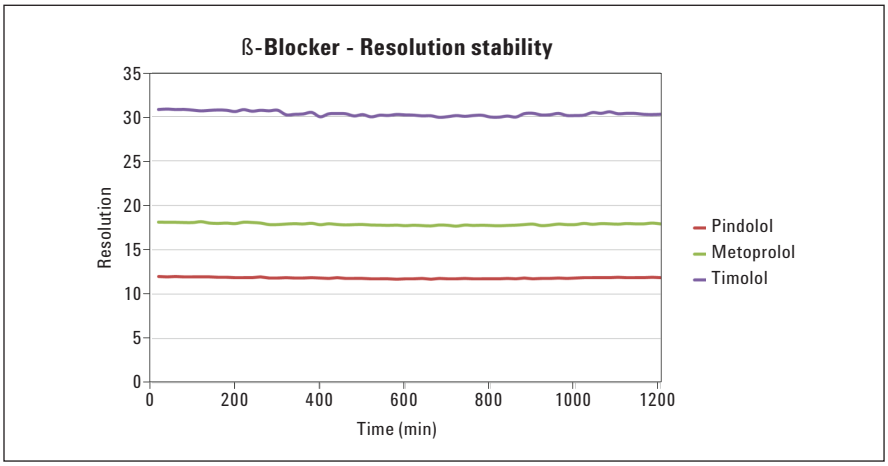

Figure 3
Resolution stability of four ß-blockers, analyzed at high pH.

High pH Analysis of Peptides

A tryptic BSA digest was analyzed using 15 mM NH_4OH as basic aqueous mobile phase (pH 10) and 90% ACN with 15 mM NH_4OH as organic mobile phase. Figure 4 shows the chromatogram of the BSA digest measured at 214 nm. Four peptides were randomly picked for the monitoring of retention time stability.

Retention times stability was monitored over a time period of 37 hours and remained stable from 0 to 2,250 minutes, see Figure 5.

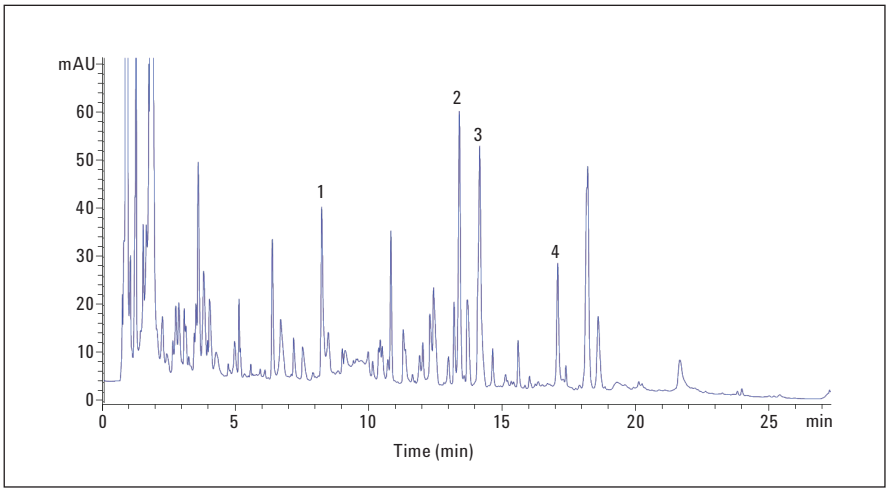

Figure 4
LC chromatogram of a tryptic BSA digest.

Conclusion

This Application Note demonstrates the feasibility of the Agilent 1260 Infinity Bio-inert Quaternary LC system for generic high pH applications. Retention time and resolution stability over a time period of 20 hours was confirmed for the analysis of ß-blockers representing small molecules. A tryptic BSA digest, as an example for biomolecules, showed retention time stability for a longer time range of 37.5 hours.

In summary, high pH mobile phases (pH 10 to 11) had no adverse effects on the stability of RT or resolution over multiple hours.

The 1260 Infinity Bio-inert Quaternary LC system as well as the Agilent ZORBAX Extend-C18 column show high stability concerning RT and resolution for high pH applications.

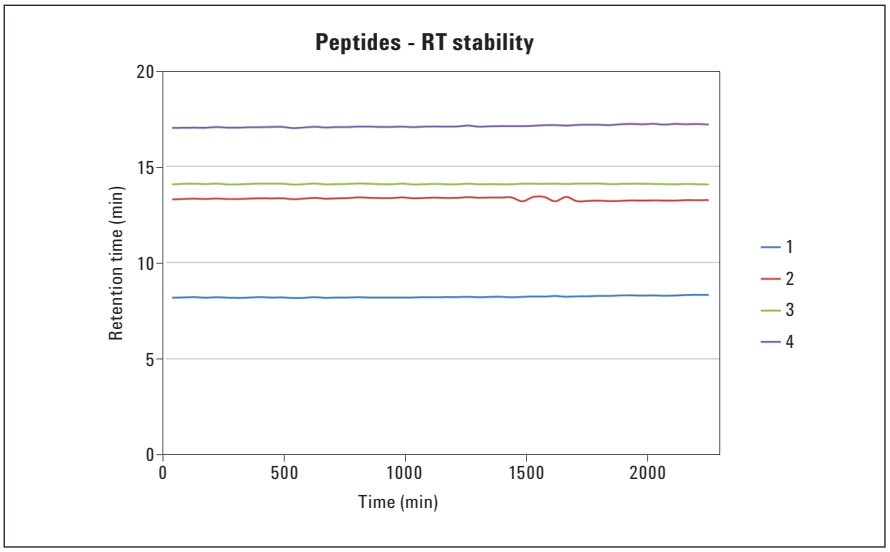

Figure 5
Retention time stability of four peptides out of a tryptic BSA digest, analyzed at high pH.

References

1.
Proof of Performance - Determination of low-metal release from the Agilent 1260 Infinity Bio-inert LC system with ICP-MS, Agilent Technologies publication 5990-9352EN, 2011.

2.
I. Peng and T. Farkas (2008). Analysis of basic compounds by reversed-phase liquid chromatography–electrospray mass spectrometry in high-pH mobile phases. *Journal of Chromatography A*, 1179, 131–144.

3.
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www.agilent.com/chem/bio-inert

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