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Improved Peak Shape and Wide Selectivity Range with ACQUITY Premier Columns

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Abstract

A family of UPLC columns designed to give improved performance for compounds that can interact with metals (metal-sensitive analytes) was recently introduced. This family includes five different general-purpose reversed phase chemistries. We investigated the selectivity differences between these columns, using a mixture of acidic, basic, and neutral analytes. The acidic analytes, hydrocortisone phosphate and dexamethasone phosphate, are shown to exhibit significant peak tailing when separated using standard stainless-steel columns. Using the new MaxPeak Premier Columns, which utilize MaxPeak High Performance Surface (HPS) Technology, we observed dramatic improvements in peak shape for these metal-sensitive analytes. With the range of selectivities demonstrated, stationary phases are available in MaxPeak Premier Columns to enable the development of separation methods for a variety of analyte mixtures.

Benefits

- ACQUITY Premier Columns show improved peak shape for metal-sensitive analytes
- A range of stationary phase selectivity is available in the ACQUITY Premier family of columns, with five different chemistries for reversed-phase applications

Introduction

The analysis of metal-sensitive compounds using HPLC has long posed significant challenges because of the use of metals such as stainless steel in the construction of columns and system components. Examples of compounds that interact with metal surfaces are those containing one or more phosphate and/or carboxylate groups. Interactions with metal parts have been shown to cause peak broadening and tailing, decreased peak areas and injection-to-injection variability.[1–3] To mitigate these interactions, a family of ACQUITY Premier Columns employing MaxPeak HPS Column hardware has been introduced.[4] The MaxPeak Premier Column family includes five different general-purpose stationary phases intended for reversed-phase applications: BEH C$_{18}$, BEH Shield RP$_{18}$, HSS T3, CSH C$_{18}$, and CSH Phenyl-Hexyl. We compared the selectivity differences between these stationary phases for separations of a mixture of acids, bases, and neutrals. The acids, hydrocortisone phosphate and dexamethasone phosphate, are metal-sensitive and suffer from peak tailing when separated using standard stainless-steel columns. We compared the separations obtained using both standard stainless steel and MaxPeak Premier Column hardware for each of the five stationary phases. The results demonstrate the benefits of the MaxPeak Premier Column hardware and the range of reversed-phase selectivities available in the ACQUITY Premier Column family.

Experimental

Sample Description

The test mixture contained 5 µg/mL thiourea, 300 µg/mL metoprolol tartrate, 40 µg/mL dipropylphthalate, 10 µg/mL amitriptyline, prednisone, hydrocortisone sodium phosphate, and dexamethasone sodium phosphate in 5% acetonitrile/95% 10 mM ammonium formate pH 3.00 (aq). Thiourea was used as the void volume (V_0) marker. Three consecutive injections of this mixture were made on previously unused columns.

Method Conditions:

LC Conditions:

System:	ACQUITY UPLC H-Class with CM-A and CM-Aux, PDA
Detection:	UV @ 254 nm
Column(s):	Various
Column temp.:	30 °C
Column configuration:	2.1 x 50 mm
Sample temp.:	20 °C
Injection volume:	2 µL
Flow rate:	0.5 mL/min
Mobile phase A:	10 mM ammonium formate pH 3 aq
Mobile phase B:	Acetonitrile
Gradient:	5-95% B in 5.3 min (linear)

Data Management:

Chromatography software:	Empower 3, Feature Release 4

Results and Discussion

The structures of the compounds used to assess the selectivity differences are shown in Figure 1. Two of these compounds are neutral (prednisone and dipropyl phthalate), two are bases which are protonated under the analysis conditions (metoprolol and amitriptyline), and two are the salts of acids (hydrocortisone sodium phosphate and dexamethasone sodium phosphate). The latter two compounds are metal-sensitive, giving broad, tailing peaks when separated using conventional stainless-steel columns. This is evident in the chromatograms shown in Figure 2B, which were obtained using standard ACQUITY UPLC Columns. When using ACQUITY Premier Columns, narrow, symmetrical peaks are achieved for these compounds, as shown in Figure 2A. Compared to the standard columns, the ACQUITY Premier Columns gave 41–72% lower tailing factors for these two metal-sensitive compounds. These differences were found to be statistically significant at >95% confidence using t-tests. In addition, the MaxPeak Premier Column gave larger peak areas, ranging from 5–53% greater than those observed for the standard columns. Except for the 5% difference, these differences were also found to be statistically significant using t-tests.
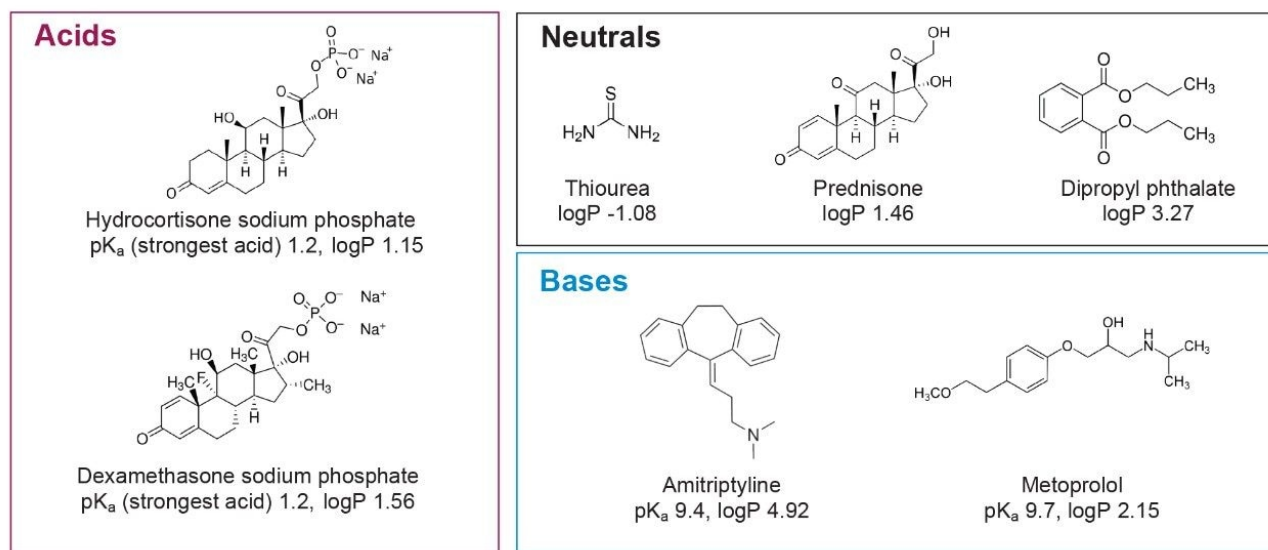

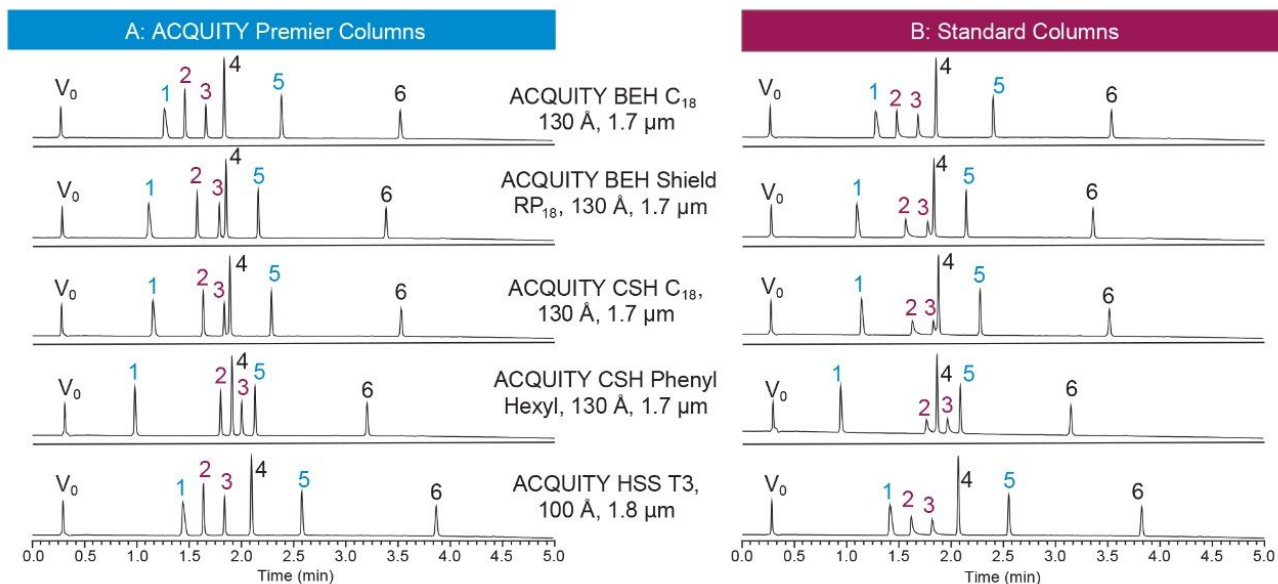Figure 1. Structures and properties of the test compounds.

Figure 2. Comparison of separations achieved using MaxPeak Premier vs standard columns. Peak identification: V_0 : thiourea, 1: metoprolol, 2: hydrocortisone phosphate, 3: dexamethasone phosphate, 4: prednisone, 5: amitriptyline, 6: dipropyl phthalate.

The chromatograms shown in Figure 2 demonstrate significant differences in retention and selectivity among the different stationary phases. Table 1 shows the chemical and physical properties of each of the five stationary phases tested, as these attributes play an important role in understanding these differences. The HSS T3 column gives the greatest retention for the neutral and basic compounds. This is attributed to the high surface area of the silica particles used to make this stationary phase and the lower C_{18} surface concentration of the T3 bonded phase.[5] The BEH Shield RP_{18}, CSH C_{18}, and CSH Phenyl-Hexyl columns show the least retention for the basic compounds. This is attributed to the presence of the embedded carbamate group in BEH Shield RP_{18}[6] and the charge modifiers in the CSH stationary phases.[7] The CSH columns also show the greatest retention of the acidic compounds, which is due to the presence of the positive surface charge on these materials. The CSH Phenyl-Hexyl column, with its alkylphenyl bonded phase, shows a change in the elution order for dexamethasone phosphate (peak 3) and prednisone (peak 4).

Table 1. Chemical and Physical Properties of the Stationary Phases

	BEH C$_{18}$	BEH Shield RP$_{18}$	CSH C$_{18}$	CSH Phenyl-Hexyl	HSS T3
Unbonded particle properties					
Particle chemistry	Hybrid	Hybrid	Hybrid	Hybrid	Silica
Surface area (m^2/g)	185	185	185	185	230
Pore volume (cm^3/g)	0.7	0.7	0.7	0.7	0.7
Average pore diameter (Å)	130	130	130	130	100
Bonded phase properties					
Chemical structure	C$_{18}$	Embedded carbamate	C$_{18}$	Phenyl-hexyl	C$_{18}$
Surface concentration (µmol/m^2)	3.1	3.2	2.3	2.3	1.6
Charge modifier	No	No	Yes	Yes	No
Endcapped	Yes	Yes	Yes	Yes	Yes

Conclusion

These results demonstrate the improved peak shapes obtained for metal-sensitive analytes that were enabled using ACQUITY Premier Columns. These improvements were achieved without any changes to the mobile phase, with a simple ammonium formate pH 3 buffer used as the aqueous mobile phase. We also highlighted the range of selectivities available in the ACQUITY Premier Column family, which includes five different reversed-phase stationary phases that vary in chemical and physical properties. This family of columns provides a useful set of stationary phases for method development, particularly for separating mixtures containing one or more metal-sensitive compounds.

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