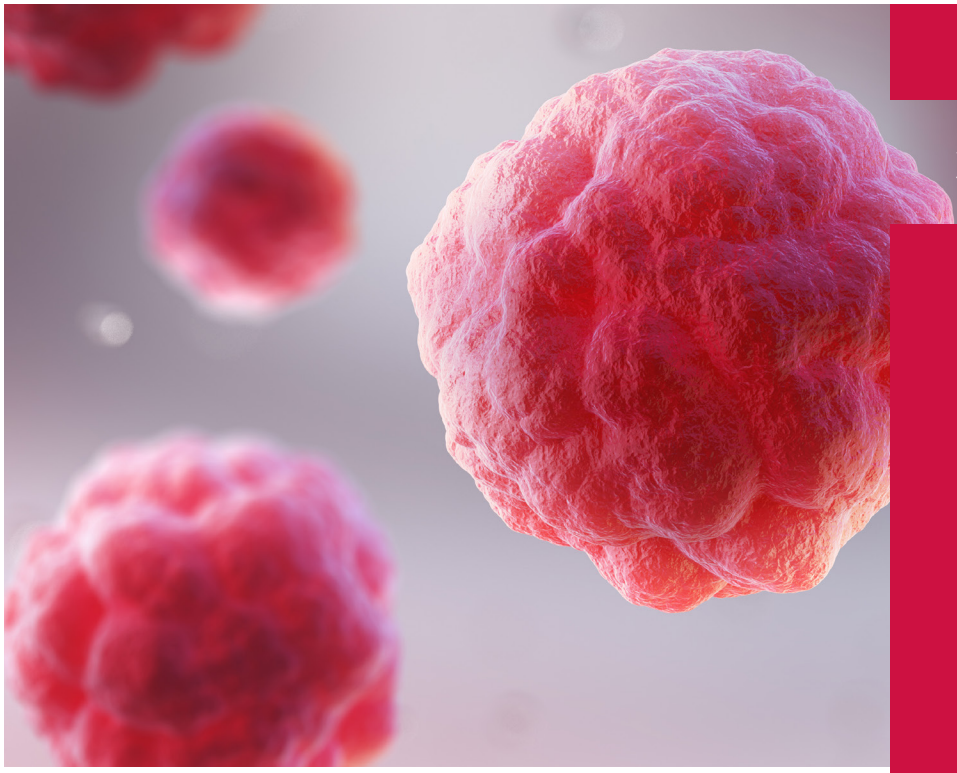

APPLICATION BRIEF

Liquid Chromatography

Improved HPLC Separation of Steroids Using a Polar End Capped LC Column

Introduction

Prednisolone, Prednisone and Cortisone are commonly used steroids to treat a range of inflammatory conditions and autoimmune disorders.[1] These synthetic derivatives of hydrocortisone were developed and approved for medicinal use as early as the 1940's.[2] American chemists first identified Cortisone as having a therapeutic benefit in the treatment of rheumatoid arthritis and was it commercialized by Merck in 1948.[3] The first commercially feasible synthesis of prednisone was carried out in 1955 in the laboratories of the Schering Corporation.[4]

A C18 HPLC column can be used for the analysis of these synthetic steroids, but they are not well retained, and resolution is incomplete. This application brief will look at the differences in chromatography between the Quasar C18 and AQ phase chemistries for the analysis prednisolone, prednisone and cortisone, Figure 1.

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Experimental Conditions

Method Parameters

All HPLC method parameters are shown in Table 1.

Table 1. HPLC method parameters

Quasar C18	150 mm	4.6 mm	5 µm	N9308802
Quasar AQ	150 mm	4.6 mm	5 µm	N9308841
Mobile Phase	H_20 0.1% formic acid: ACN, 20:80			
Flow Rate	1mL/min			
Temp	20 °C			
Wavelength	254 nm			
Injection Vol.	5 µl			
Analyte	i. Prednisolone ii. Prednisone iii. Cortisone			

Solvents and Samples

All solvents were HPLC grade and samples were filtered using a 0.45 µm nylon filter, P/N 02542880.

Results and Discussion

As these synthetic steroids contains several polar functionalities consequently they do not exhibit strong retention using a standard C18 HPLC column, Figure 2A. In the past this problem could be addressed using ion pair regents, but such methodology is not compatible with MS detectors which are common place today due to the sensitivity gains they offer.

The drive for improved retention of polar compounds without the addition of additives led to the development of "AQ" type phases. There are two general approaches to the bonded phase chemistry of AQ columns; to either employ a polar or hydrophilic endcapping or embed a polar entity, such as an amide, within the alkyl chain. Both options improve the retention of polar compounds, under reverse phase HPLC conditions.

The same three steroids were then analysed using a Quasar AQ column, Figure 2B. Both increased retention and improved resolution is observed when compared to the analysis using the Quasar C18 column; t_R (cortisone) now 11.5 minutes as opposed to just under 4 minutes. The polar end-capping of the Quasar AQ stationary phase offers enhanced retention of these polar compounds due to increased dipole-dipole interactions of the analyte with the stationary phase. Excellent peak shape is also yielded as the optimised ligand bonding process provides exceptional surface coverage eliminating unwanted secondary silanol interactions. Further improvement in resolution of corticosteroids (i) and (ii) can be achieved by moving from a 5 µm AQ column to a 3 µm AQ column.

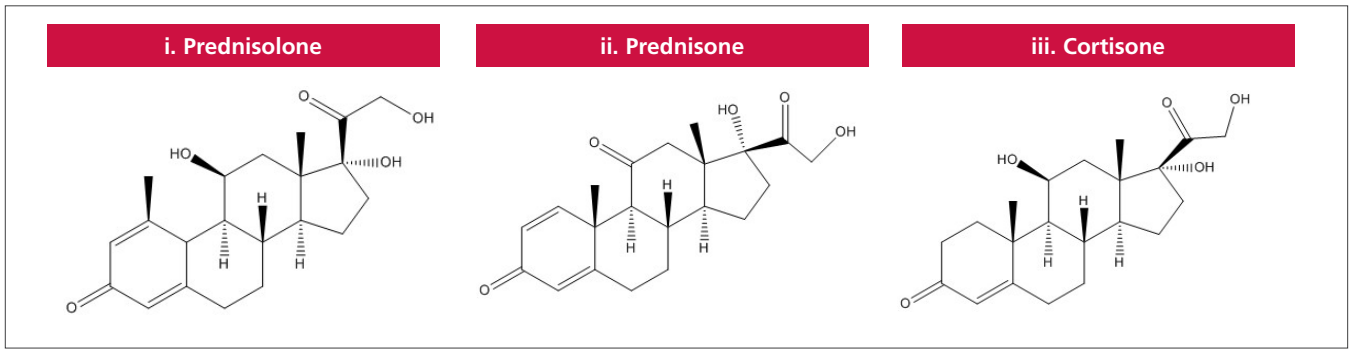

Figure 1. Chemical structures of Prednisolone, Prednisone and Cortisone steroids.

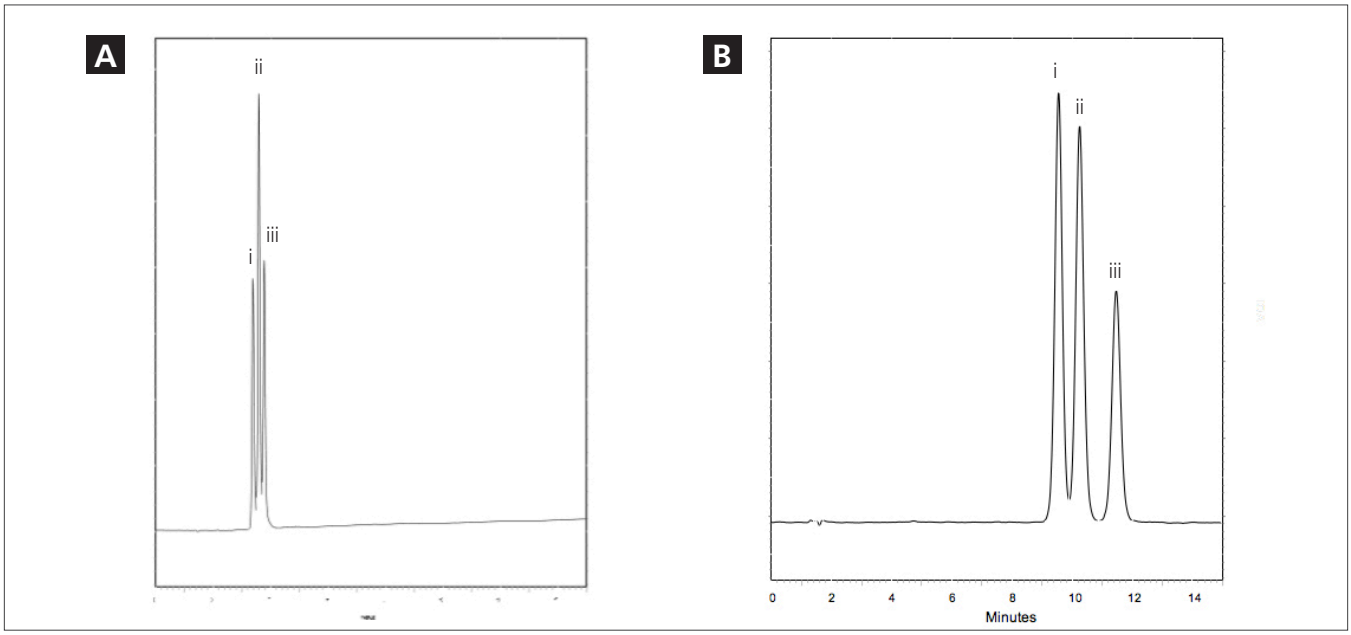

Figure 2. HPLC analysis of Prednisolone, Prednisone and Cortisone steroids A) on a Quasar C18 column 150 x4.6 mm 5µm, B) on a Quasar AQ column150 x 4.6 mm 5 µm.

Conclusion

- Analysis of these three corticosteroids can be completed using a C18 column, but due to their similar polar structure, resolution is not baseline and retention is poor.
- The polar nature of these compounds makes them a suitable for analysis using the Quasar AQ column.
- Increased retention and separation are achieved with the Quasar AQ column, compared to the C18 phase.
- Because of the polar end capping, there are more sites for interaction of the stationary phase and the polar analytes thus improving separation.

References

1. "Prednisolone". The American Society of Health-System Pharmacists. Archived from the original on 23 December 2016.
2. Kim K, Roh JK, Wee H, Kim C (2016). Cancer Drug Discovery: Science and History. Springer. p. 169.
3. Thomas L. Lemke; David A. Williams (2008). Foye's Principles of Medicinal Chemistry. Lippincott Williams and Wilkins. pp. 889
4. Fischer, Janos; Ganellin, C. Robin (2006). Analogue-based Drug Discovery.

Consumables

Phase	Length (mm)	I.D. (mm)	µm	Part
Quasar AQ	250	4.6	5	N9308840
Quasar AQ	150	4.6	5	N9308841
Quasar AQ	100	4.6	5	N9308842
Quasar AQ	50	4.6	5	N9308843
Quasar AQ	150	4.6	3	N9308844
Quasar AQ	100	4.6	3	N9308845
Quasar AQ	50	4.6	3	N9308846
Quasar AQ	150	3.0	3	N9308847
Quasar AQ	100	3.0	3	N9308848
Quasar AQ	50	3.0	3	N9308849
Quasar AQ	150	2.1	3	N9308850
Quasar AQ	100	2.1	3	N9308851
Quasar AQ	50	2.1	3	N9308852
Quasar AQ	100	4.6	1.7	N9308853
Quasar AQ	50	4.6	1.7	N9308854
Quasar AQ	100	3.0	1.7	N9308855
Quasar AQ	50	3.0	1.7	N9308856
Quasar AQ	100	2.1	1.7	N9308857
Quasar AQ	50	2.1	1.7	N9308858
Quasar AQ Guard Cartridge (3/pack)	10	3	5	N9308986
Quasar AQ Guard Cartridge (3/pack)	10	3	3	N9308987
Quasar Guard Cartridge Holder	-	-	-	N9306876
Quasar C18 Method Validation Kit, 250x4.6 mm (3/pack)	250	4.6	5	N9300940
Quasar C18 Method Validation Kit, 150x4.6 mm (3/pack)	150	4.6	5	N9300941

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