APPLICATION BRIEF

Liquid Chromatography

Warfarin Analysis Using a Quasar AQ UHPLC Column

Introduction

Today, Warfarin is the most widely used anticoagulant in the world, used to thin the blood and prevent clots.[1] It was discovered by chance when in the 1920's cattle in the US were found to be bleeding to death having eaten mouldy hay from sweet clover crops.[2] However, the exact identity of the substance causing the haemorrhaging was to remain unknown for many years.

Over the coming years studies of the spoiled hay eventually led to the extraction of a compound which was later named dicoumarol. It was observed that this dicoumarol could not act as an anticoagulant on its own. It was only after it was metabolised by fungi that it exhibited anticoagulant properties. This explained why only spoiled hay caused the outbreak in the cattle.

After further research, the synthesis of a more potent anticoagulant from dicoumarol, warfarin, was produced. Warfarin first commercial use was as a rat poison in 1948, followed by license for human use in 1953.

This application brief illustrates the analysis of warfarin, Figure 1, using the Quasar AQ liquid chromatography phase.

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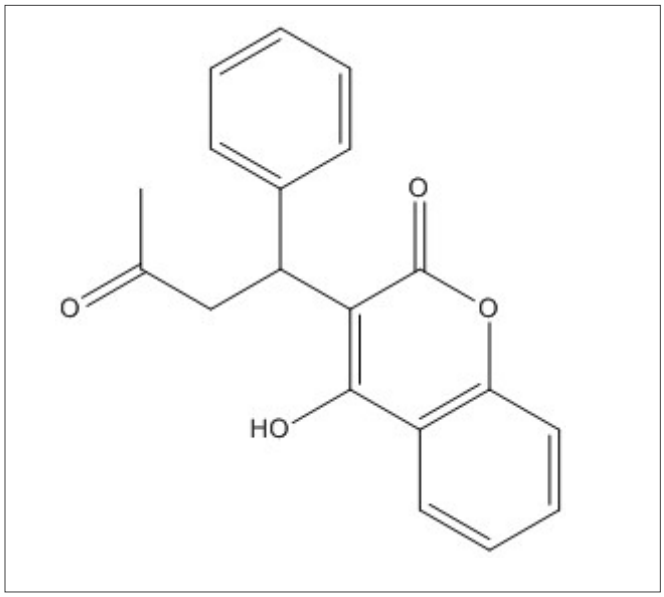

Figure 1. Chemical Structure of Warfarin.

Experimental Conditions

Method Parameters

All HPLC method parameters are shown in Table 1.

Table 1. HPLC Method Parameters.

Quasar AQ	50 mm	2.1 mm	1.7 µm	N9308857
Mobile Phase	40:60, H_2O 0.1% formic acid: ACN			
Flow Rate	0.25 mL/min			
Temp	25 °C			
Wavelength	220 nm			
Analyte	Warfarin			

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 warfarin contains several polar functionalities it consequently does not exhibit strong retention using a standard C18 HPLC column. Use of ion pair reagents does improve retention but renders the method incompatible with MS detectors.

This common problem of poor retention of polar entities lead to the development of several "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 within the alkyl chain. Both options improve the retention of polar compounds, under reverse phase HPLC conditions, without the need to use ion pair reagents.

For the analysis of warfarin, Figure 2, the Quasar AQ column was used. This phase has a polar endcapping and offers retention of warfarin due to the 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.

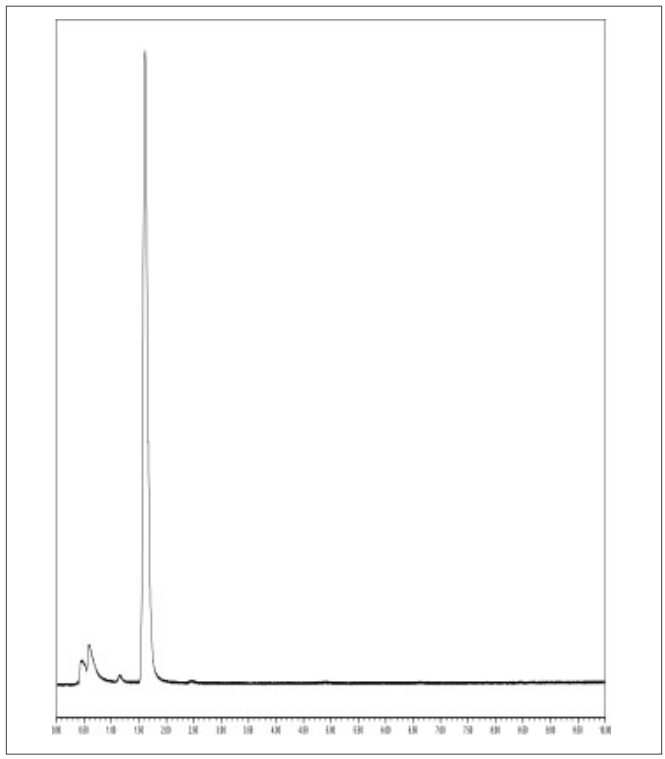

Figure 2. HPLC analysis of Warfarin.

Conclusion

- The polar nature of warfarin makes it a suitable for analysis using the Quasar AQ column.
- Quasar AQ phase yields retention under reverse phase conditions as a consequence of the polar end capping on the stationary phase providing sites for interaction with the polar entities within Warfarin.
- No ion pair reagents are required to gain this retention; thus, the method is suitable for MS or UV detection.

References

1. "The discovery of Heparin and Warfarin, D. Wardrop, D. Keeling, Oxford Haemophilia and Thrombosis Centre, Oxford Radcliffe Hospitals, Oxford, UK https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2141.2008.07119.x
2. http://eatonwarfarin.com/2016/02/03/history-of-warfarin/

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

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