Introduction

A cyano HPLC column (USP-L10) is specified in the Chromatographic Purity method for warfarin published in the USP Formulary (USP 27 - NF 22) [1]. Results are presented here that demonstrate that Eclipse XDB-CN meets or exceeds the USP method requirements for this analysis. An analysis of a generic warfarin tablet is also included.

Experimental

An Agilent 1100 HPLC system consisting of a binary pump, autosampler, and diode array detector (DAD) was used. Detection was at 260 nm. Injections of 2 µL were made with the autosampler.

All separations were performed isocratically at 1.5 mL/min and 25 °C with a premixed eluent of 68:32:1 water:acetonitrile:glacial acetic acid.

Warfarin was purchased from Sigma, Inc. (Catalog No. A2250 - 98% min)

Warfarin-related Compound A was purchased as a USP Reference Standard from USP (Catalog No. 71910)

Standard Solution: Prepared using ¼ of each of the amounts specified in the USP method. Specifically, accurately weighed quantities of about 6 mg warfarin and 6 mg of warfarin-related compound A were transferred to a 50-mL volumetric flask. One mL of 0.1N NaOH was added along with 12.5 mL of methanol to dissolve the compounds, and then diluted to the mark with water. Instead of diluting by a factor of 50 using two dilution steps, 2 µL was injected instead of 50 µL (twice the amount injected on column than specified in the USP method, which provides larger peaks).

Sample Solution: No production warfarin was available for checking chromatographic purity (other than the Sigma standard (98% min)) so a generic tablet of warfarin (0.223 gm), containing nominally 5 mg of warfarin and manufactured by Barr Laboratories, was dissolved by placing it in 6.25 mL of 25:75 methanol:water

Highlights

- The Eclipse XDB-CN (L10) column exceeds all the performance requirements for the USP method for chromatographic purity of warfarin.
- Eclipse XDB-CN can also be used to separate related compounds.
- Eclipse XDB-CN provides fast, high-resolution separations for purity analyses.
and ultrasonicating for 30 minutes. The resulting concentration of warfarin was the same as that specified for the final test solution concentration in the USP method for chromatographic purity. This solution was then filtered through a 0.2 µm syringe filter before injection into the chromatograph.

Results and Discussion

Figure 1 shows the separation of the standard solution of warfarin and related compound A on the Eclipse XDB-CN. This mixture is well separated using the elution conditions specified in the USP Formulary (USP 27 - NF 22) for chromatographic purity of warfarin, which specifies an L10 (cyano) column.

<table>
<thead>
<tr>
<th>Experimental conditions</th>
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<tbody>
<tr>
<td>Column: Eclipse XDB-CN (L10-USP)</td>
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<tr>
<td>4.6 mm x 150 mm, 5 µm</td>
</tr>
<tr>
<td>p/n 993967-905</td>
</tr>
<tr>
<td>Eluent: 32:68:1 ACN:Water:Glacial acetic acid</td>
</tr>
<tr>
<td>Flow: 1.5 mL/min</td>
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<tr>
<td>Temperature: 25 °C</td>
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<tr>
<td>Detection: UV 260 nm</td>
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<tr>
<td>Injection volume: 2 µL</td>
</tr>
</tbody>
</table>

Figure 1. HPLC separation of warfarin and warfarin related compound A on an Eclipse XDB-CN (L10 - USP) column.

The chromatographic purity method also specifies that the resolution between warfarin and warfarin-related compound A should not be less than 3 and that the relative retention times should be 1.0 and about 1.2 for warfarin and its related compound A, respectively.

Table 1 indicates that the Eclipse XDB-CN (L10) column easily meets these requirements for the chromatographic purity method. The relative retention time (RRT) and resolution for the warfarin-related compound A exceed the requirements specified in the method.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RRT</th>
<th>Rs</th>
</tr>
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<tbody>
<tr>
<td>Warfarin</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Warfarin Related Compound A</td>
<td>1.24</td>
<td>5.45</td>
</tr>
</tbody>
</table>
Figure 2 shows the analysis of a generic warfarin tablet (nominally 5 mg) manufactured by Barr Laboratories. Figure 2A shows the analysis in full scale with a peak height of 67 mAU for the warfarin peak. Evidence of warfarin-related compound A is not apparent in Figure 2A. When this chromatogram is magnified 67 times so that the chromatogram is 1 mAU full scale, a very slight rise and fall of the baseline is observed close to the retention time for the warfarin-related compound A. However, the size of this “peak” (if it is real) is at the signal-to-noise (S/N) limit so no conclusion can be reached regarding the presence or absence of this compound.

![Figure 2](image)

**Figure 2.** HPLC analysis of a generic warfarin tablet on an Eclipse XDB-CN (L10 - USP) column.

**Conclusions**

The Eclipse XDB-CN (L10) column exceeds all the performance requirements for the USP method for chromatographic purity of warfarin. In addition, the results for the Eclipse XDB-CN column suggest that it might also be suitable for the analysis of warfarin and warfarin-related compound A in warfarin tablets.

**Reference**

1. USP Method “Warfarin Sodium”, USP27 - NF22.
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