RESULTS

The synthesized 6α,9α-difluoroprednisolone 17α,21-methylorthobutyrate eluted at a retention time of 8.51 minutes.

The mass spectrum of the synthesized compound (Figure 2) exhibited a pseudomolecular ion at 481.2394 m/z. The molecular mass of 6α,9α-difluoroprednisolone 17α,21-methylorthobutyrate is 480.23. The mass-to-charge ratio obtained for the synthesized compound indicates that the desired product was obtained since it matched with the expected m/z.

A reaction between a 17α,21-hydroxy steroid and the appropriate orthoester using acid catalysis, was carried out using green chemistry principles, to synthesize the corresponding 17α,21-steroid orthoester (Figure 1).

AIMS

To develop an LC-HRMS method for the separation and identification of orthoesterification reaction products and to establish whether the desired 17α,21-steroid orthoester was obtained.

METHOD

LC-HRMS analysis was conducted using Thermo LTQ Orbitrap Discovery system. A fused core Ascentis® Express C18 column (2.7µm; 100mm by 2.1mm) was used.

The sample was synthesized at the Department of Pharmacy, of the University of Malta, using green chemistry principles and prepared using acetonitrile as solvent (1µg/mL).

The mobile phase was composed of acetonitrile and 0.1% formic acid, using a gradient elution (Table 1). The injection volume was set as 5µL. Liquid chromatography was performed at 40°C using a PDA detector set at 240nm. The FTMS mode was set at a resolution of 30,000. The instrument was operated in positive ionization mode using a data type centroid of 100-1000 m/z.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Acetonitrile (%)</th>
<th>Formic acid 0.1% (%)</th>
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<tr>
<td>0.0</td>
<td>5</td>
<td>95</td>
</tr>
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<td>5</td>
<td>95</td>
</tr>
<tr>
<td>12.5</td>
<td>5</td>
<td>95</td>
</tr>
</tbody>
</table>

LC-HRMS Analysis of Synthesized Steroids

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CONCLUSION

An innovative, fast, gradient LC-HRMS method was developed for the analysis of the synthesized steroid, which led to the elution of the compound within a short time. The sample preparation method is simple and accomplished within a short time. To the authors’ best knowledge, the mass spectrometry analysis of 6α,9α-difluoroprednisolone 17α,21-methylorthobutyrate is not available in literature.

INTRODUCTION

A reaction between a 17α,21-hydroxy steroid and the appropriate orthoester using acid catalysis, was carried out using green chemistry principles, to synthesize the corresponding 17α,21-steroid orthoester (Figure 1).

Figure 1: 6α,9α-difluoroprednisolone 17α,21-methylorthobutyrate

Figure 2: Mass spectrum of the 17α,21-steroid orthoester

Table 1. The gradient method used for the liquid chromatography.