Analysis of synthesized isomeric steroids using liquid chromatography-high resolution mass spectrometry

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INTRODUCTION

The characterization of synthesized products to determine the identity of the compounds produced and whether the reaction was indeed successful is a very important step in the research and development process.

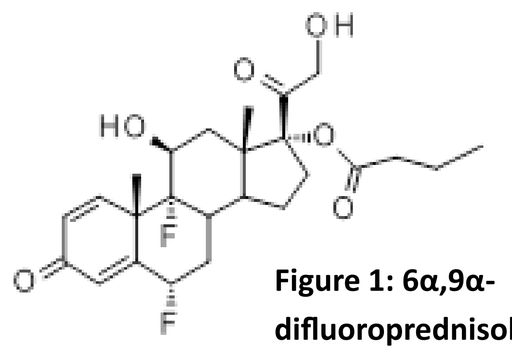

Figure 1: 6α,9α-difluoroprednisolone 17-butyrate

AIMS

To develop a simple and fast analytical method using liquid chromatography-high resolution mass spectrometry (LC-HRMS) to establish whether the desired 17-butyrate (Figure 1) was synthesized

METHOD

Sample preparation

The sample, consisting of a mixture of compounds, was dissolved in HPLC grade acetonitrile at a concentration of 1 µg/ml.

Liquid chromatography

Analysis was conducted using Thermo LTQ Orbitrap Discovery system coupled to LC. A fused core Ascentis® Express C18 column with a particle diameter of 2.7µm (100mm by 2.1mm) was used. The temperature was set at 40°C and the PDA detector at 240nm. An injection volume of 5 µl was used.

Gradient elution using LSMS grade acetonitrile and aq. 0.1% formic acid (v/v) as mobile phase.

The percentage composition of acetonitrile was changed from 5% (v/v) at 0.0 minutes, to 100% (v/v) at 8 minutes and 5% (v/v) at 11.5 minutes respectively.

HRMS Analysis of Synthesized Steroids

The MS was set at a resolution of 30,000 using FTMS mode. The instrument was operated in positive ionization using the centroid mode. Data was acquired for the 100-1000 amu mass range.

RESULTS

LC-HRMS analysis led to the separation and detection of two compounds with a retention time of 7.04 min and 7.46 min respectively.

The mass spectrum of the isolated products (Figure 2) showed that both compounds had a molecular ion of 467.224 m/z, indicating the presence of two structural isomers.

The molecular mass of the desired product, i.e. 6α,9α-difluoroprednisolone 17-butyrate, is 466.22. The result obtained using mass spectrometry indicated that one of the synthesized products may correspond to the desired product.

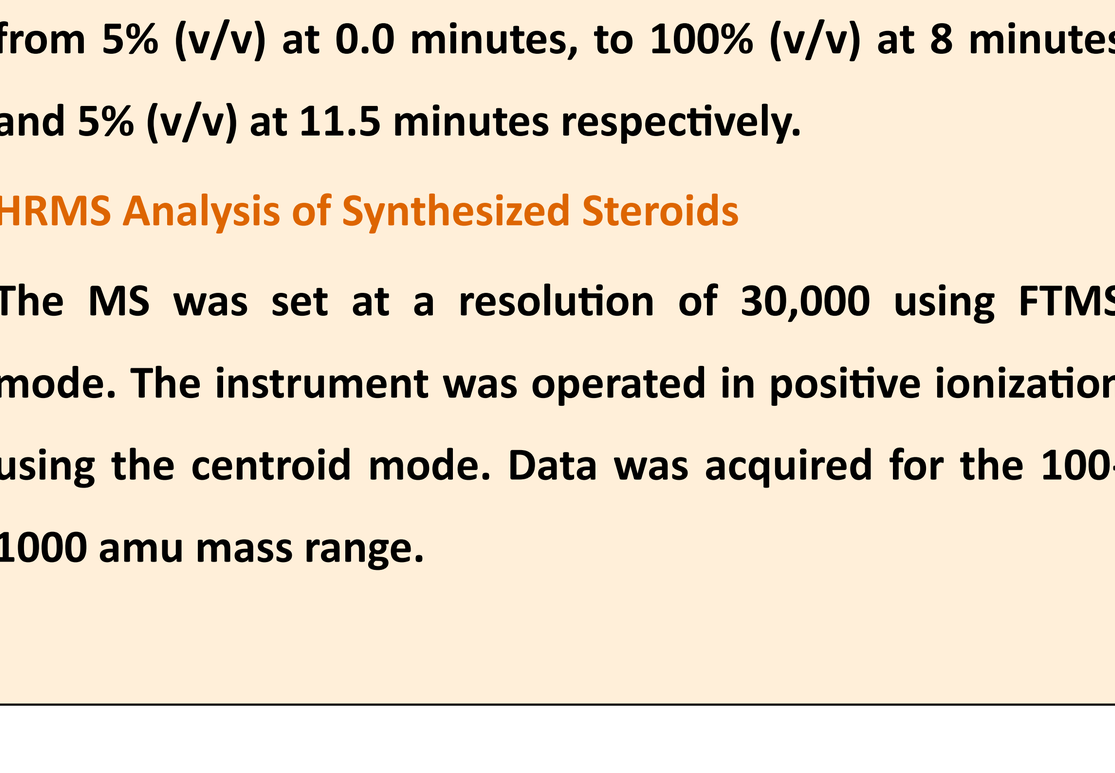

Figure 2: Mass spectrum of the resultant esterified steroids obtained via hydrolysis

CONCLUSION

The developed LC-HRMS method was fast, required minimal sample preparation time and successfully separated two isomers. The mass-to-charge ratio of the two isomers corresponds to the expected value for the desired product. The synthesized compounds may be attributed to the desired 6α,9α-difluoroprednisolone 17-butyrate and its isomer 6α,9α-difluoroprednisolone 21-butyrate.