Development of a semi-preparative HPLC method for diflupredne

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INTRODUCTION

In 2008, the steroid difluprednate was the first steroid to be approved by the Food and Drug Administration for the post surgery treatment of both pain and inflammation of the eyes.[1-3]

Synthesis often leads to the formation of impurities which may or may not be structurally similar to the desired product. This induces difficulties in purifying the product for instrumental analysis, for example NMR, in order to elucidate its structure. Purification of samples using semi-preparative HPLC provides a means to obtain highly pure samples in large enough amount to be used for further analysis.[4]

AIMS

To develop a semi-preparative HPLC method for the separation and purification of difluprednate and impurities obtained during synthesis.

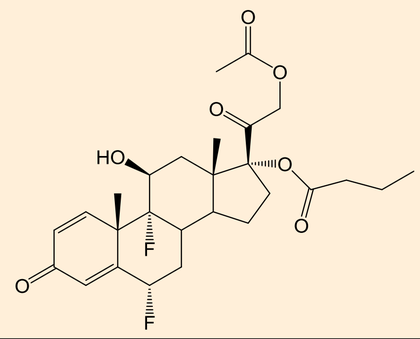

Figure 1. Difluprednate

METHOD

Materials and Equipment

A Lab Alliance Prep Pump coupled to a UV detector was used for the semi-preparative HPLC. The column used was a Supelco Discovery HS C18 (25cm x 10mm, 10µm). Samples were prepared at a concentration of 10mg/ml by dissolving the crude product in the organic solvent used for the mobile phase. All solvents used were of HPLC grade.

Semi-preparative Liquid Chromatography method

An isocratic method was used for the purification of difluprednate and impurities produced during synthesis.

Two different mobile phase compositions were assessed to establish the conditions at which the best separation is obtained. The mobile phase compositions used were methanol/water (6:4) and acetonitrile/water (6:4) respectively.

The flow rate, wavelengths and injection volume used were the same for both methods. The flow rate was set at 4mL/min. Analysis was conducted at four different wavelengths 220nm, 240nm, 280nm and 600nm respectively. The injection volume was 500µL.

RESULTS

The semi-preparative HPLC method for separating the impurities from difluprednate was improved by changing the mobile phase composition from methanol/water (Figure 2a) to acetonitrile/water (Figure 2b). The separation of difluprednate from its impurities was successful.

The method using acetonitrile/water was found to be shorter compared to the method using methanol/water, with a run time of 12 minutes compared to over 30 minutes with methanol.

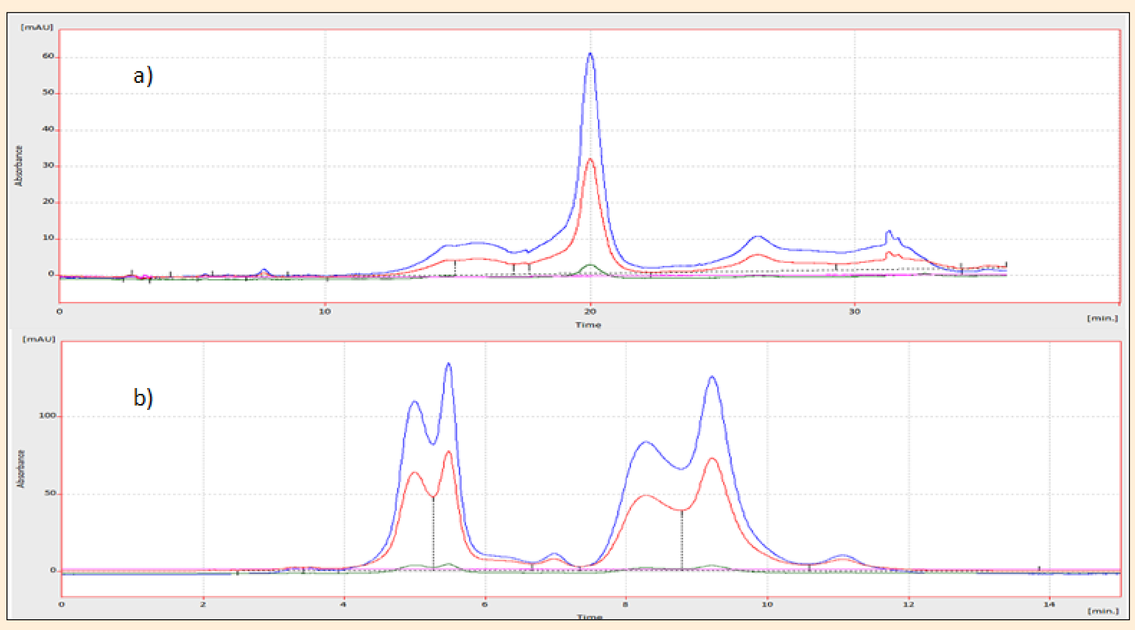

Figure 2: Separation at different wavelengths (220nm, 240nm, 280nm, 600nm) using the mobile phase composition a) methanol/water (6:4) b) acetonitrile/water (6:4)

CONCLUSION

A relatively fast semi-preparative high performance liquid chromatographic method using isocratic elution has been developed for the purification of difluprednate for further instrumental analysis such as NMR, UV and IR.

References

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