# A Qualitative and Quantitative Method for Difluprednate using High Performance Liquid Chromatography

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## INTRODUCTION

Monitoring and determination of impurities during synthesis of active pharmaceutical ingredients (APIs), require the use of various analytical methods. Initially not all the chemical structures of impurities are known therefore various methods should be developed to allow the reliable distinction between one chemical structure from another[1]. Since most of the impurities generated during a reaction are similar to the product, different compounds may co-elute, masking the presence of some impurities[1-3].

## AIMS

To develop an analytical method, using HPLC, to detect and quantify difluprednate and the impurities produced during the synthesis of this steroid.

## METHOD

### Materials and equipment

HPLC grade acetonitrile and water, sodium phosphate dibasic and orthophosphoric acid were bought from Sigma-Aldrich (Steinhelm, Germany). The RP-HPLC analysis was conducted using an Agilent® 1260 equipped with a Kinetex® C18 100A 150x4.60mm column with 5µm particle size.

### Chromatographic conditions

The mobile phase was composed of acetonitrile and 10mM phosphate buffer solution (pH 6) at 50:50v/v. Temperature was kept at 40°C. UV wavelength was set at 240nm and a flow rate of 1ml/min was used. The injection volume used was 5µL.

### Sample preparation

A 2mg/ml stock solution was prepared by dissolving difluprednate in acetonitrile and diluted to the required concentrations using acetonitrile.

### Method validation

Validation was conducted according to the ICH guidelines. Linearity was determined by carrying out three replicates of six concentrations. Specificity was assessed by running a blank. The limit of detection and limit of quantification were calculated using the standard deviation of the response and the slope of the calibration curve.

## RESULTS

### Linearity

The peaks obtained were well defined and symmetrical. The calculated coefficient of determination, $r^2$, was 0.99999, indicating that there is a good linear relationship between the peak area and the concentration (Figure 1). The method was found to be linear in the range of 0.0125mg/ml to 1.2mg/ml (Figure 2).

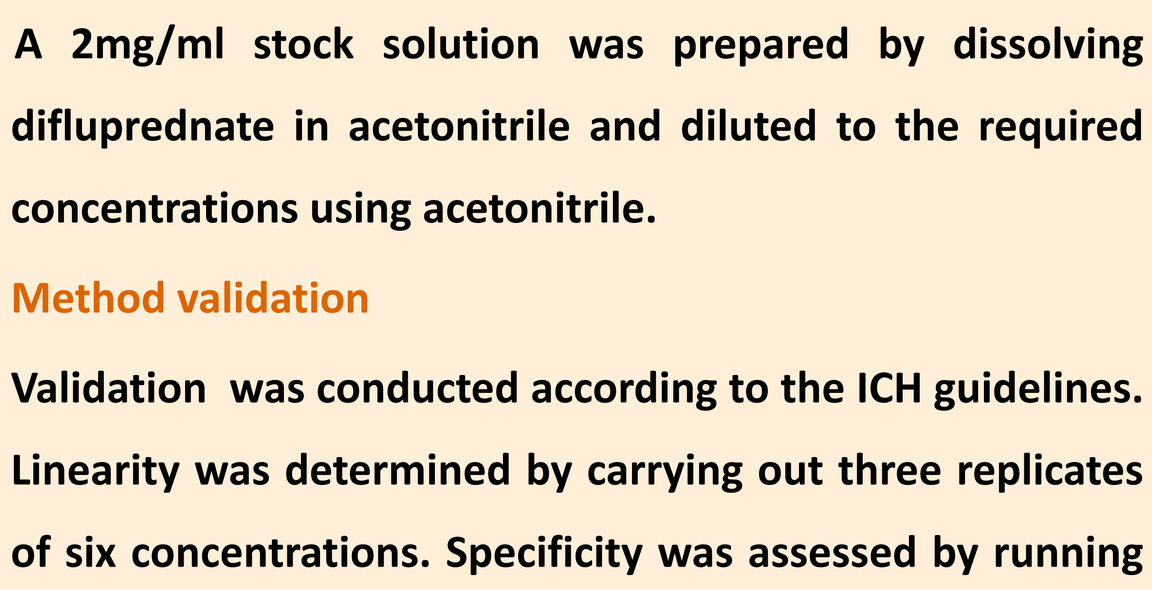


Figure 1: The regression line for difluprednate

### Specificity

When a blank was run, no peak was visible at the retention time of difluprednate, confirming that this method is specific to difluprednate.

### Limit of detection (LOD) and quantification (LOQ)

The LOD was found to be 0.0025mg/ml and the LOQ was 0.0077mg/ml.

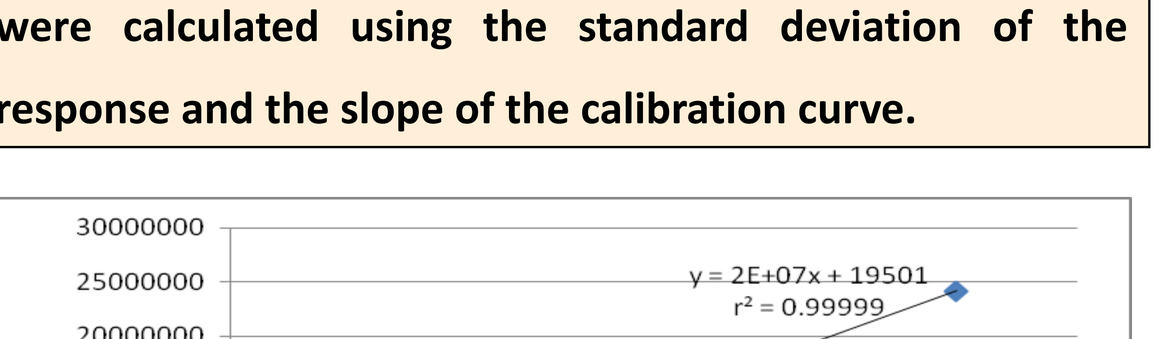


Figure 2: The chromatogram obtained for difluprednate at a concentration of 1.2mg/ml

## CONCLUSION

The developed method was found to be linear and specific to difluprednate. The validation of the method for precision, recovery and ruggedness is now in progress.

### References

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