Estimation of Cortisone Acetate in Pharmaceutical Anti-inflammatory Drugs by HPLC-UV Technique

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Abstract: The HPLC-UV System was used to assay Cortisone acetate in Oral Tablets So; compare the bioavailability of two types of Cortisone acetate; commercial formulations and Cortisone acetate analar grade as a test formulation. Cortisone acetate concentrations were analyzed by HPLC-UV System at (λ=254 nm). The separation was achieved by using the Ion Pac Ercus C18 RP-Column; 5µm, 4.6×250 mm. The mobile phase consisted of water/acetonitrile (55:45). The study of bioequivalence between the two Cortisone acetate formulations were assessed by calculating peaks height. The standard Cortisone acetate eluted at a flow rate of 1.0 ml/min. The method was found to be linear in the range (0.5 to 2.5) µg/ml (n = 5) with R² ≥ 0.9991, also, the recoveries were range within 99-100%. The detection limit of quantification (LLOQ) was 0.0790µg/ml and lower limit of detection (LOD) 0.02610 µg/ml showing average intra assay and inter-assay coefficients of ± RSD % about 0.522 %. The results of recoveries, ± RSD, and statistical parameters obtained in this study. The accurate, precise and sensitive refers to validation method for determination of Cortisone acetate in anti-inflammatory Pharmaceutical drugs.

Keywords: Cortisone acetate, Oral Tablets, formulation, and HPLC-UV System

1. Introduction

Cortisone Acetate is description into glucocorticoid and the Biological description shows glucocorticoid and mineral corticoid activities. Delays ovo-implantation. Shows immunosuppressant, anti-inflammatory and analgesic effects in vivo [1]. Orally active a white or practically white, odorless, crystalline powder, Solúble in DMSO and insoluble in water, It is stable in air [2], it’s a designated chemically name [2-[(8S, 9S, 10R, 13S, 14S, 17R)-17-Hydroxy-10, 13-dimethyl-11-dioxo-1, 2, 6, 7, 8, 9, 12, 14, 15, 16-Decahydrocyclopenta[a] phenanthren-17-yl]-2-oxoethyl] acetate, molecular Formula (C₂₁H₂₆O₂) and Molecular weight is 402.49gm mol⁻¹. Cortisone Acetate naturally occurring glucocorticoids (hydrocortisone, cortisone and cortisone acetate) [3, 4], which also have salt-retaining properties, are used as replacement therapy in adrenocortical deficiency states [5]. Anti-inflammatory are proven medications for both human and veterinary [6]. They are also used for their potent anti-inflammatory effects in disorders of many organ systems. Intractable to adequate trials of conventional treatment: Seasonal or perennial allergic rhinitis Bronchial asthma Contact dermatitis Atopic dermatitis Serum sickness Drug hypersensitivity reactions[7-9].

There are a several methods for quantitative determination of Cortisone Acetate. The most interesting field of IC Chromatography application is the analysis of pharmaceutical drugs, for example, to control the quality of the pharmaceutical drugs itself and in clinical analyses to study the effects and the evolution of the pharmaceutical drugs in human body [10, 11]. The analysis of inflammatory is also important due to the wide ranging application of these drugs [12].

Due to structural similarities of some impurities with Cortisone acetate make separation of the individual components within Cortisone acetate potentially difficult. However, these otherwise hydrophilic compounds can be separated by performance liquid chromatography, reversed phase- HPLC at wavelength, 254 nm that assist in accentuating the small hydrophobicity differences. It’s contain chromophores, making UV detection with high

Figure 1: Structure of Cortisone acetate congeners

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sensitivity [13-15]. Cortisone derivatization with Acetate be
determined by changing the wavelength that depends on
absorbance morality factors before sample derivatization
measurements. The Cortisone acetate are separated on an
Ion Pac Ercus C18 RP-Column; 5µm, 4.6×250 mm, the
mobile phase consists of a mixture of a water /acetonitrile
(55:45), and quantified by UV detection. This method,
although effective, is an indirect detection method, which
requires additional preparation time and reagents for
derivatization [16-18].

Aim of study
The main aim for this study was to find differences between
the Cortisone acetate of two commercial type’s formulations
(Oral Tablets) and standard Cortisone acetate high purity as
a test formulation. The method assaying for this drugs were
assessed by calculating peaks height. So, the results for all
tests refers to validate and success this method.

2. Materials and Method
All solvents and reagents were of analytical grade unless
indicated otherwise, and all experiments were performed
with deionized water (18.2 Ω·cm) resistivity at 25 °C [19].

Equipments
Chromatography experiments were carried out by a HPLC-
UV chromatography consisting of:
LKB Bump 2150 –HPLC, Bromma
Ion Pac Ercus C18 RP-Column; 5µm, (250×4.6 mm id) (P/N
11051194 L) from European was chosen for some drugs
separation.

Metrohme Electric injection valve with 100 µL loop fitted
in.

A PD 303 UV detector single beam (Japan) equipped with
an 18 µl flow cell (Helma. UK.) Data logger Lab JackU12
acquisitions (Ocean control/ Australia).

Personal computer supplied with modify software programs
/ cvi programs UV.
Printer (EPSON-L210 / Japan).
pH meter (Hana- Italy).

Reagents and standards:
Acetonitrile for HPLC grade, BDH Chem. LTD
Cortisone acetate liquid and analar Cortisone acetate as
standard Sigma-Aldrich German.

Water was obtained by following purification in a deionized
water system.

From a stock solution containing 25.0 µg/ml Cortisone
acetate in mixture of water /acetonitrile (55:45), a standard
curve was prepared at the concentration of 2.5, 5.0, 7.5, 10.0
and 12.5 µg/ml in mixture of water /acetonitrile (55:45). For
standardization, 100 mL of the standard solutions of
Cortisone acetate were transferred to glass tubes at room
temperature, the concentration range of standard curve was
diluted five times in mobile phase and the corresponding
solution was submitted to chromatographic analysis at 0.5,
1.0, 1.5, 2.0 and 2.5 µg/ml of Cortisone acetate [20, 21].

Procedure
All of chromatographic measurements were carried out
using HPLC-UV chromatography at room temperature and
pressure of 80 bar [22, 23], which consisting LKB pump
2150-HPLC pumping the eluent at 1 ml/min. Cortisone
acetate samples or standard were manually injected with
Metrohme electronic injection valve fitted with 100 µl loop
in eluent of a mixture of a water/acetonitrile (55: 45 v/v) at
room temperature [24]. Ion Pac Ercus C18 RP-Column;
5µm, (250×4.6 mm id) (P/N 11051194 L) was used as a
separation column [25]. APD 303 UV-Vis detector single
beam spectrophotometer (Japan) equipped with 18 µl flow
cell (Helma UK) was used to measure the absorbance signal
at 254 nm of the separated species. A data logger lab jack-
Ocean control/ Australia was sending a data to Personal
computer and printer were handling the data of the HPLC-
UV system. The peaks height of a symmetrical peaks is
contemporary to the Cortisone acetate concentration of
standards and sample concentrations [26, 27].

<table>
<thead>
<tr>
<th>Table 1: Method Parameters</th>
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<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>Description Column</td>
</tr>
<tr>
<td>System Suitability Requirement</td>
</tr>
<tr>
<td>Isocratic Mobile phase</td>
</tr>
<tr>
<td>Test sample</td>
</tr>
<tr>
<td>Detection System</td>
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<tr>
<td>Maximum Wavelength</td>
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<tr>
<td>Flow Rate</td>
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<tr>
<td>Temperature</td>
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<tr>
<td>Pressure Background</td>
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<tr>
<td>Retention Time</td>
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<tr>
<td>Run Time</td>
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<tr>
<td>Injection Volume</td>
</tr>
</tbody>
</table>
3. Results and Discussion

1. Effect of column type, eluent concentration and Retention time on Cortisone acetate separation

Ion Pac Ercus C18 RP-Column; 5µm, (250 × 4.6 mm id) column was recommended as a suitable and efficient separation column for Cortisone acetate [28]. Which can be detect by using UV-Vis detector at λmax 254 nm with mixture of eluent consist water/acetonitrile (55:45) which can be freshly prepared [29].

Figure 2 shows that the column has high efficiency to separate Cortisone acetate, the linear gradient is 7 minutes for each injection and one peak appearance in chromatogram. The distinct peak cause of good method sensitivity to determine Cortisone acetate [30, 31].

2. Effected Column temperature on the separation:
The Metrohme 690 IC system supply with column temperature evaluating in the range 25-45 °C in five degree steps. As expected, increasing the column temperature decreased retention time and led to good baseline for the separation chromatogram of the standards and samples [32].

Figure 2: Chromatograms Calibration curve of Cortisone acetate

3. Method performance (linearity, Reproducibility and Detection Limits):

Under the established conditions listed in Table 1, a method of the standard calibration was used to obtain the calibration curve for Cortisone acetate, by plotting the concentration versus the peaks height of asymmetrical peaks. It is linear over the range (0.5-2.5) µg/ml Cortisone acetate. Table 3 lists the R² and slope of the curve, which are 0.9991 and 13.2 respectively (figure 4).

The reproducibility of the method was estimated by injection of a 1.0, 1.5 and 2.0µg/ml represented standard Cortisone acetate and two commercial Cortisone acetate drugs into eluent. Excellent RSD% for retention time (tR) and peaks height were obtained as shown in Table 2 and 3. Lower limit of detection (LLOD) and quantitation (LLOQ), LLOD=3.3 SD/S and LLOQ=10 SD/S are the concentrations that give the signal to noise ratio of 3:1 or 10:1 respectively. This can be detected and verified by the divided of standard deviation of response (SD) by the slope of calibration curves (S) [33, 34] By using the single-sided student’s test method (at the 95% confidence limit) for five consecutive injections of 1.5µg/ml of Cortisone acetate sample and standard [35, 36], the values of LLOD and LLOQ were 0.02610µg/ml and 0.07909 µg/ml respectively.

Table 2: The reproducibility of peaks height and tR of Cortisone acetate

<table>
<thead>
<tr>
<th>Representative samples and drugs (µg mL⁻¹)</th>
<th>Peaks height (mm)</th>
<th>± *RSD%</th>
<th>Retention Time (tR, minutes)</th>
<th>±*RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>11</td>
<td>± 0.517</td>
<td>7</td>
<td>± 0.151</td>
</tr>
<tr>
<td>1.5</td>
<td>20</td>
<td>± 0.522</td>
<td>7</td>
<td>± 0.111</td>
</tr>
<tr>
<td>2.0</td>
<td>27</td>
<td>± 0.894</td>
<td>7</td>
<td>± 0.122</td>
</tr>
<tr>
<td>2.5 µg mL⁻¹ for Drugs (1)</td>
<td>11</td>
<td>± 0.511</td>
<td>7</td>
<td>± 0.094</td>
</tr>
<tr>
<td>2.5 µg mL⁻¹ for Drugs (2)</td>
<td>11</td>
<td>± 0.488</td>
<td>7</td>
<td>± 0.177</td>
</tr>
</tbody>
</table>
To evaluate the accuracy of the HPLC Method which used to determination of cortisone acetate, a recovery experiments were performed on three representative standards and two commercial drug samples. Standard additions method (Figures 5 and 6) was used for all of these determinations in order to avoid all the possible interferences [37, 38]. Table 4 summarized all of these studies. A good agreement between the results was obtained which clearly indicated that HPLC Method which used to several applications for determination of cortisone acetate in different pharmaceutical drugs.
This work described a Metrohme 690 semi-automated HPLC system equipped with UV detector for Cortisone acetate determination in pharmaceutical drugs. This developed method offer simple, inexpensive and needs only a very small volume of the sample and using a UV detector makes this system very specific due to one peak in the chromatogram. In this application there is no need for high sensitivity since the pharmaceutical drugs of Cortisone acetate have a very low concentration.

### References