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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 of 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 belinear in the range (0.5 to 2.5) µg/ml (n = 5) with $R^2 \geq 0.9991$, also, the recoveries were range within 99-100%. The detection limit of quantification (LLOQ) was 0.07909µg/ml and lower limit of detection (LLOD) 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, Soluble in DMSO and insoluble in water, It is stable in air [2], it's a designated chemically name [2-[(8*S*, 9*S*, 10*R*, 13*S*, 14*S*, 17*R*)-17-Hydroxy-10, 13-dimethyl-3, 11-dioxo-1, 2, 6, 7, 8, 9, 12, 14, 15, 16-Decahydrocyclopenta[*a*] phenanthren-17-yl]-2-oxoethyl] acetate, molecular Formula ($C_{23}H_{30}O_6$) 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].

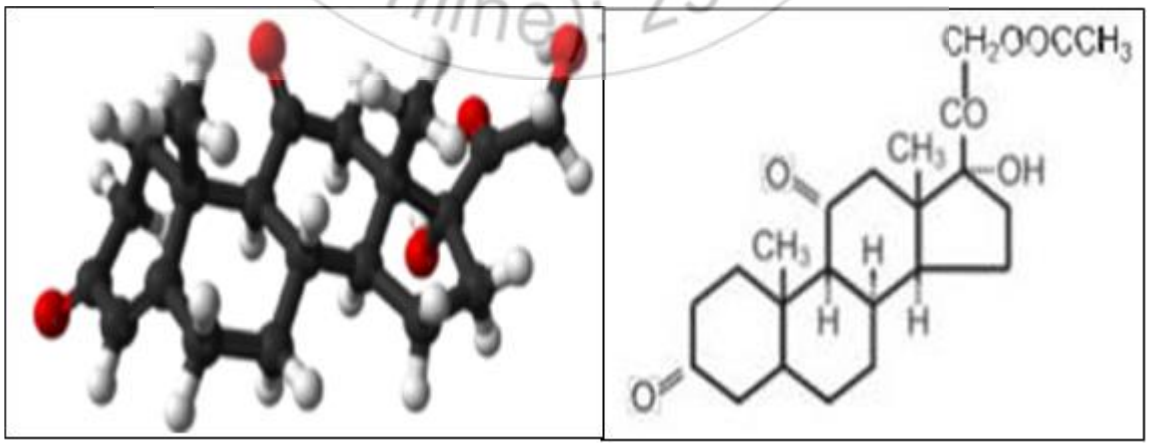


Figure 1: Structure of Cortisone acetate congeners

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

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 ºC [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.

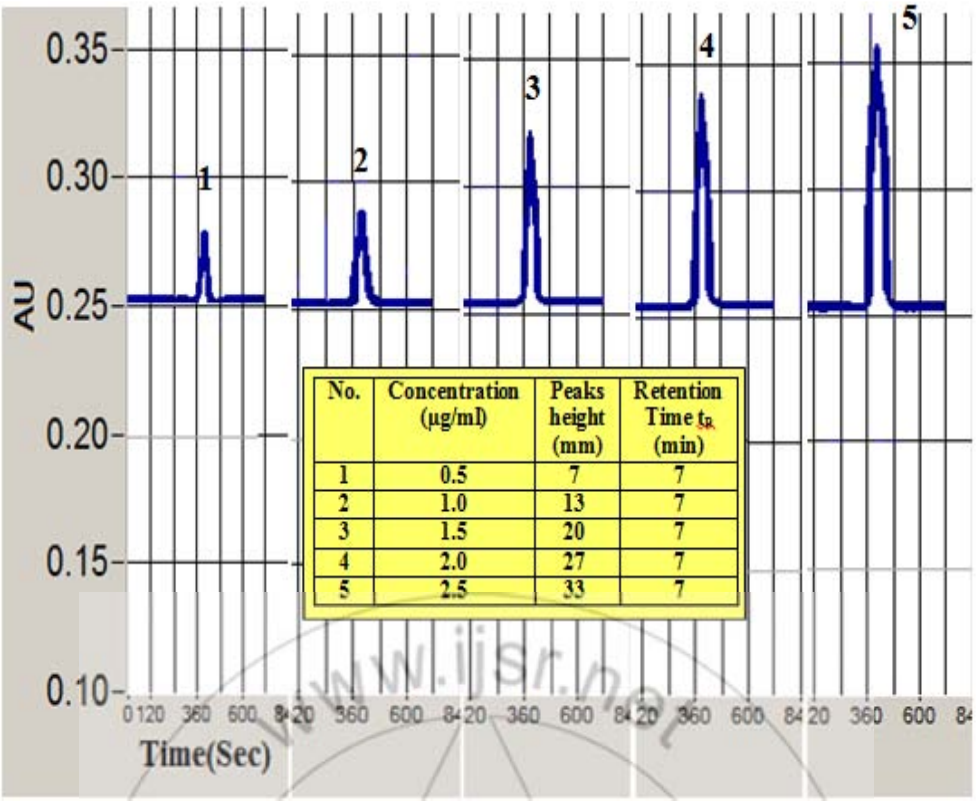
From a stock solution containing 25.0 µg/ml Cortisone acetate in mixture of water /acetonitrile (55:45), a standard curve was prepared at the concentration of 2.5, 5.0, 7.5, 10.0 and 12.5 µg/ml in mixture ofwater /acetonitrile (55:45). For standardization, 100 mL of the standard solutions of Cortisone 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 corresponding to the Cortisone acetate concentration of standards and sample concentrations [26, 27].

Table 1: Method Parameters

Parameters	Conditions
Description Column	Ion Pac Ercus C18 RP-Column; 5µm, (250×4.6 mm id) (P/N 11051194 L)
System Suitability Requirement	USP Tailing Factor @ 5 % From average Peaks Height 1.22 Plates/Column ≥1742.22
Isocratic Mobil phase	water /Acetonitrile (55/45)
Test sample	Cortisone acetate diluted in the mobile phase
Detection System	UV detection
Maximum Wavelength	254 nm
Flow Rate	1.0 mL / min.
Temperature	At room temperature
Pressure Background	80 Bar
Retention Time	7 min.
Run Time	12 min
Injection Volume	100 µL



No.	Concentration (µg/ml)	Peaks height (mm)	Retention Time t_R (min)
1	0.5	7	7
2	1.0	13	7
3	1.5	20	7
4	2.0	27	7
5	2.5	33	7

Figure 2: Chromatograms Calibration curve of Cortisone acetate

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 λ_{max}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 ºC 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].

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 R^2 and slope of the curve, which are 0.9991and 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 (t_R) 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 t_R of Cortisone acetate

Representative samples and drugs (µg mL^{-1})	Peaks height (mm)	± *RSD%	Retention Time (t_R) minutes	±*RSD%
1.0	11	±0.517	7	±0.151
1.5	20	±0.522	7	±0.111
2.0	27	±0.894	7	±0.122
2.5µg mL^{-1} for Drugs (1)	11	±0.511	7	±0.094
2.5µg mL^{-1} for Drugs (2)	11	±0.488	7	± 0.177

Accuracy:

Table:3: Regression statistics of the proposed method with LLOD, LLOQ, Intercept and Slope

R^2	0.9991
Standard Error	0.3830
Standard Error Estimate	0.3651
Intercept	0.2
Slope	13.2
LLOD µg mL^{-1}	0.02610
LLOQ µg mL^{-1}	0.07909
MDL(standard) µg mL^{-1} ($SD \times t_{95\%}$) at n= 5-1	0.28982
MDL(sample) µg mL^{-1} ($SD \times t_{95\%}$) at n= 5-1	0.29100

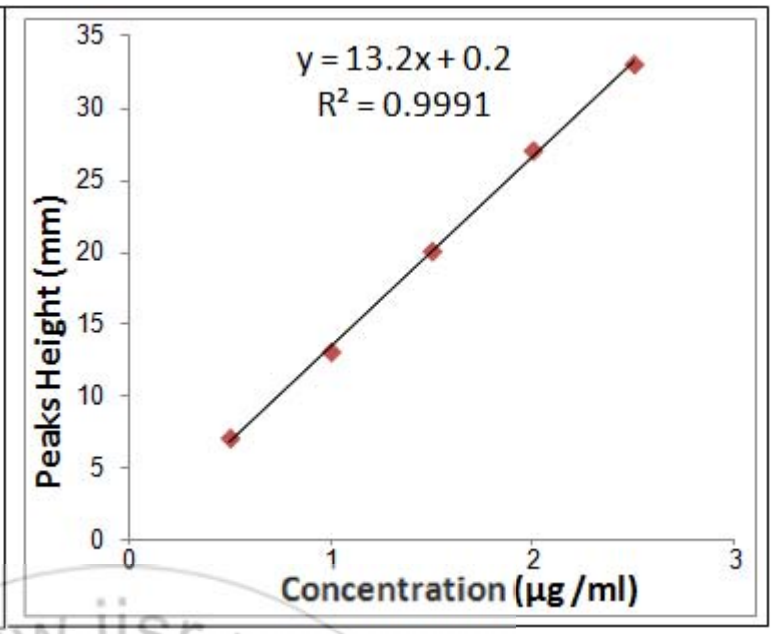


Figure 4: Standard Calibration graph of Cortisone acetate Standard

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.

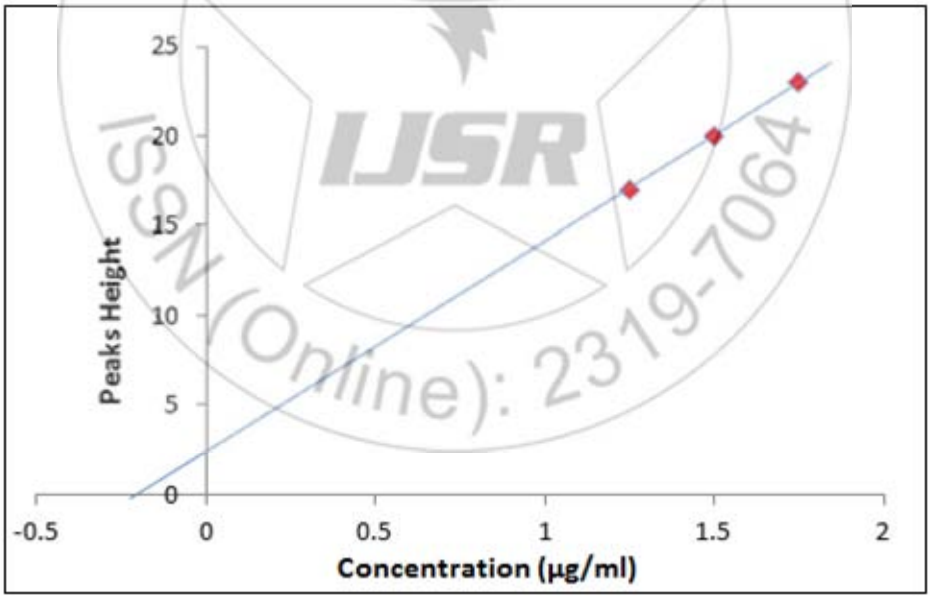


Figure 5: Standard additions for Cortisone acetate determination

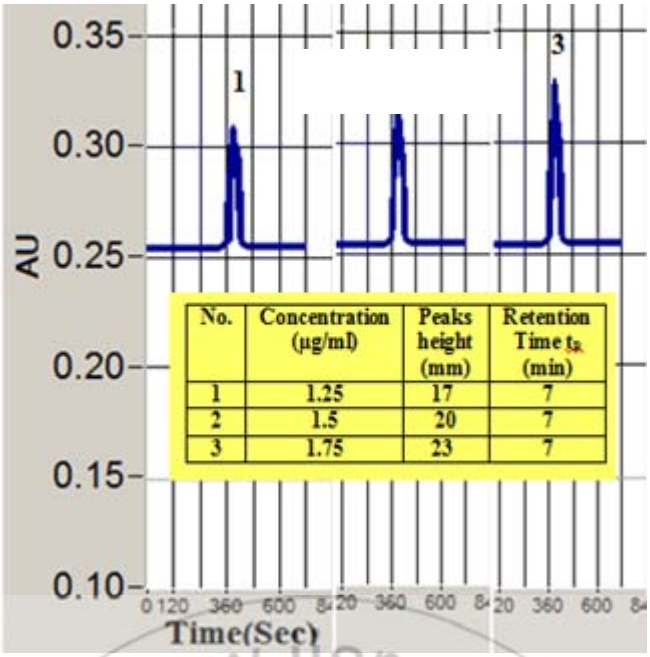


Figure 6: peaks of Standard addition Method

Table 4: Cortisone acetate recoveries obtained by HPLC-UV system

Claimed Conc. (µg mL^{-1})	Found conc. (µg mL^{-1})	Recovery± RSD
1.0	1.00	100 ±0.517
1.5	1.49	99.3 ± 0.522
2.0	1.98	99 ±0.498
2.5µg mL^{-1} for Drugs (1)	2.49	99.6 ± 0.459
2.5µg mL^{-1} for Drugs (2)	2.50	100 ±0.501

Precision

Precision of method, reported as % RSD, was estimated by measuring repeatability (intra-day assay) for five replicate injections for all concentrations of Cortisone acetate. The intermediate precision (inter-day variation) were also studied for two days using an intermediate concentration solution of Cortisone acetate. The Intra-day average recoveries were in the range (99-100) and Inter-day average recoveries (98-110) which thought to be an acceptable result [39, 40, 41]. The obtained results are summarized in Table 5

Table 5: Intra and inter-day precision and accuracy of standard analysis (n = 5).

Claimed conc. (µg mL^{-1})	Intra-day		Inter-day	
	Found (µg mL^{-1})	±Recovery % RSD	Found (µg /ml)	± Recovery % RSD
0.5	0.5	100 ± 0.478	0.5	100 ± 0.511
1.0	1.00	100 ±0.517	1.10	110± 0.200
1.5	1.89	99.1 ± 0.522	1.55	101 ± 0.541
2.0	1.94	99 ±0.894	2.0	100 ± 0.220
2.5	2.50	100 ± 0.459	2.85	94 ± 0.547
2.5 µg/ml Drug (1)	2.89	99.6 ± 0.459	2.89	99.2 ± 0.214
2.5 µg/ml Drug (2)	2.50	100 ±0.501	2.5	100 ± 0.595

4. Conclusion

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.

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