World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 8, Issue 5, 2019 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article

# SIMULTANEOUS ESTIMATION OF HYDROCORTISONE AND KETOCONAZOLE IN TABLET DOSAGE FORM BY UV-SPECTROSCOPY

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**Received on: 09-04-2019; Revised and Accepted on: 18-05-2019**

***ABSTRACT***

***T****wo simple, precise and accurate methods were developed and validated for the Simultaneous estimation of Hydrocortisone and Ketoconazole in the combined dosage forms. The first method involves the usage of Simultaneous equation for the determination and the second method employed is the Multi component mode. For both the methods the wavelengths selected were 245nm and 270nm which are the absorbance maxima of Hydrocortisone and Ketoconazole respectively. The drugs obeyed Beer's law in the concentration range of 5-30µg/mL for hydrocortisone and 10-60 µg/mL for Ketoconazole. The results of the analysis were validated statistically and by recovery Studies.*

***KEYWORD:*** *Hydrocortisone,Ketoconazole, Multi component mode.*

## INTRODUCTION

**H**ydrocortisone (HYDRO) is chemically (1S,2R10S, 11S,14R,15S,17S)-14,17-dihyroxy-14-(2-hydroxyacetyl)-2,15-di methyltetracyclo[8.7.0.0^{2,7}heptadec-6-en-5-one. HYDRO belongs to Antiinflammato ry Agents. Structure of HYDRO was shown in figure 1[1].

Ketoconazole (KETO) is chemically 1-[4-(4-{2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy]phenylpiperazin-1-yl]ethan-1-one. It is used as Antifungal agents. Structure of KETO was shown in figure 2 [2].

Literature studies revealed that there is no method till date developed for the estimation of these two drugs in the combined dosage form and hence a simple, precise and an accurate UV-Spectrophotometric method has been developed in the present study.

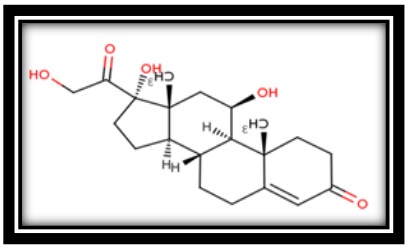

**Fig. 1: Hydrocortisone**

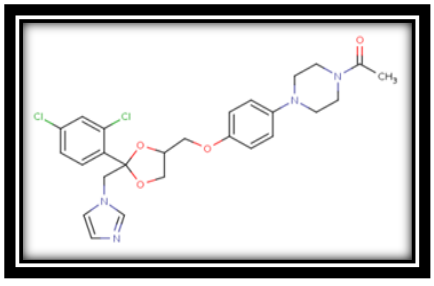

**Fig. 2: Ketoconazole**

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**DOI: https://doi.org/10.5281/zenodo.3236709**

## EXPERIMENTAL

**Material and Reagents**:

Analytically pure HYDRO was kindly provided by Hetero Laboratory, and KETO was provided by Mylan Laboratory, as gift samples. Analytical grade methanol was purchased from Merck & Co. Glasswares used in each procedure were soaked over night in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. Water was purchased from Merck, India. Triple distilled water is used for all purpose.

**Instrumentation:**

UV- system (Model-1700 with UV Probe Software) Manufacturer is Shimadzu. SHIMADZU-1700+ UV-Visible double beam spectrophotometer with a fixed slit width 1nm and 1cm matched quartz cells was used for all the spectral measurements.

**Pharmaceutical Preparation:**

The commercial pharmaceutical preparation of the combination is Ketocon + plus. The content includes Hydrocortisone 1%w/v and Ketoconazole 2 %w/v.

**Selection of Common Solvent:**

The selection of solvent is made after assessing the solubility of both the drugs in various solvents. Methanol is selected as the common solvent and the spectral characteristics were studied out using this solvent.

**Preparation of Standard Drug Solution:**

Standard Hydrocortisone of 10mg and Ketoconazole of 10mg was accurately weighed and transfered in to a 100 ml volumetric flask. Add small amount of methanol in volumetric flask and dissolve the drug in to it and then adjust the volume up to 100ml. This solution from 100ppm of concentration and then prepare other dilution.

**Selection of Analytical concentration Range:**

From the standard stock solution of hydrocortisone (100µg/ml), appropriate dilutions of 5ppm, 10ppm up to 30ppm are prepared.

From the standard stock solution of Ketoconazole (100µg/ml), appropriate dilutions of 10ppm, 20ppm up to 60ppm are prepared.

**Determination of Absorption Maxima:**

By appropriate dilution of two standard drug solutions with methanol, solutions containing 10 µg/mL of HC and 10 µg/mL of KE were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both the drugs. HC and KE showed absorbance maxima at 245 nm (λ1) and 270 nm (λ2) respectively (**Fig. 3**).

**Method I (Simultaneous Equation Method):**

Two wavelengths selected for the method are 245 nm and 270 nm that are absorption maxima of HC and KE respectively in Methanol. The stock solutions of both the drugs were further diluted separately with methanolto get a series of standard solutions of 5-50 µg/mL concentrations.

The absorbances were measured at the selected wavelengths and absorptivities for the drugs were determined. Concentrations in the sample were obtained by using following equations-

$$A1 = a_{x1}C_X + a_{y1}\,C_Y \quad A2 = a_{x2}C_X + a_{y2}\,C_Y$$

Where, A1 and A2 are absorbance's of mixture at 241.5nm and 271 nm respectively, ax1 and ax2 are absorptivities of HC at λ1 and λ2 respectively and ay1 and ay2 are absorptivities of SP at λ1 and λ2 respectively. Cx and Cy are concentrations of HC and KE respectively.

$$0.392 = 464.64\,C_x + 143.95\,C_y$$
$$1.331 = 27.385\,C_x + 635.832\,C_y$$

**Method II (Multicomponent Mode):**

Multicomponent mode was employed for the estimation of these drugs in the combined dosage form. For this method of analysis 245and 270 nm were selected as the sampling wavelengths. The drugs showed linearity in the range of 5- 30µg/mL for HC and 10-60µg/mL for KE. Three mixed standard solutions containing both the drugs were prepared in the ratio of 1:2 and the spectral characteristics were studied after the data is fed into the instrument. The instrument then gave the concentrations of individual drugs in the test by an inbuilt microprocessor.

**Method Validation:**

The method was validated according to ICH Q2B guidelines [8] for linearity, accuracy and precision.

***1. Linearity:*** Linearity solutions for the method were prepared from the stock solutions of both the drugs and spectral runs were carried out.

***2. Precision:*** This is studied to find out the intra and the inter day variations in the developed method. The %RSD of the three assay values at three different concentrations on same day (intraday) and on three different days (interday) values were calculated.

***3. Accuracy:*** The accuracy of this method was evaluated at three concentration levels and the recovery studies were performed by standard addition method. Percent recovery for both the methods was then calculated.

## RESULTS AND DISCUSSION

The overlain spectra of Hydrocortisone and Ketoconazole exhibit $\lambda_{max}$ of 245nm and 270nm respectively. These wavelengths were selected for both the methods. Standard calibration curves for the drugs were described with equations y=0.046x + 0.001 for HC and y=0.063x for KE for simultaneous equation method and y=0.046x+0.003 and y=0.062x+0.008 for HC and KE respectively for Multicomponent mode. The correlation coefficient for both the drugs was found to be 0.999 in both the methods.

The beer Lambert's linearity range of these drugs includes 5-30µg/mL for HC and 10-60µg/mL of SP. The results were tabulated in **table 1 and 2**. The %RSD for the intraday was found to be 0.945 for HC and 0.529 for KE. Similarly 0.70 and 1.49 are the %RSD of HC and SP respectively for interday studies for simultaneous equation method.

In the case of Multicomponent mode the %RSD for intraday was found to be 0.33 for both the drugs and %RSD for interday studies were found to be 0.82 and 0.76 for HC and KE respectively.

The %RSD for intraday and interday precision were found to be less than 2 indicating that the method is precise. The results of these were presented in **table 3.**

The accuracy was confirmed by the recovery studies and the percentage recovery was found to be in the range of 96-102% thus justifying the method. From accuracy studies it was found that the RSD was less than 2% indicating that the method is accurate. The results were presented in **table 4**.

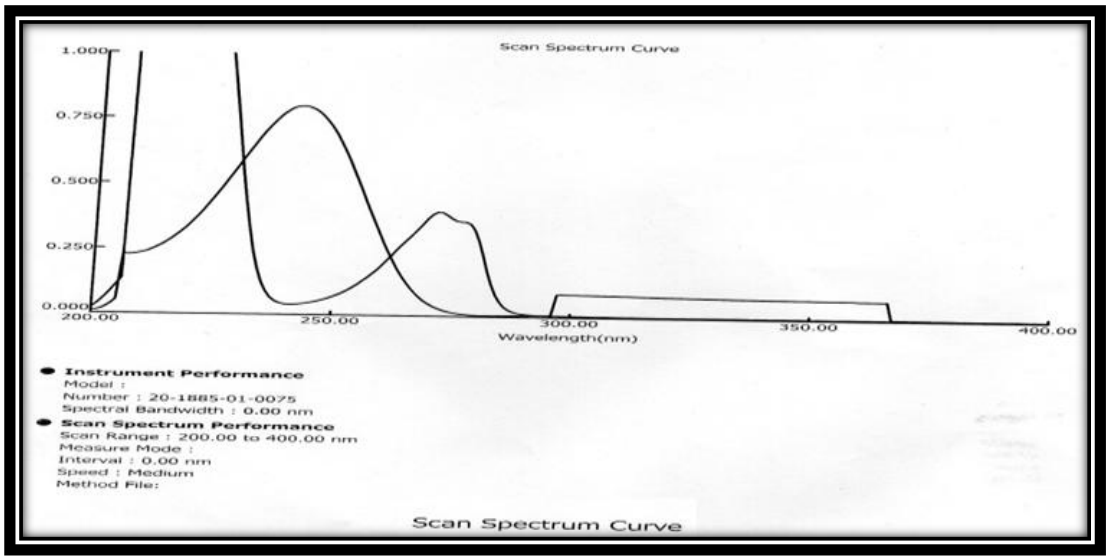


**Fig. 3: Overlain Spectrum of Hydro Cortisone (10µG/ML) and Ketoconazole (10µg/ml)**

**Table No. 1: Linear Regression analysis of Calibration curves**

| Parameters | Method I | | Method II | |
|---|---|---|---|---|
| **Drugs** | **HC*** | **KE*** | **HC*** | **KE*** |
| **Beer's law limit (µg ml$^{-1}$)** | 5 – 30 | 10 – 60 | 5 – 30 | 10–60 |
| **Correlation coefficient** | 0.999 | 0.999 | 0.999 | 0.999 |
| **Molar absorptivity (lit/mole/cm)** | 18566.55 | 14999.97 | 18485.65 | 15094.45 |
| **Sandell's sensitivity(mcg/Sq.cm/0.001)** | 0.0217 | 0.0157 | 0.0218 | 0.0156 |
| **Slope** | 0.046 | 0.063 | 0.046 | 0.062 |
| **Intercept** | 0.001 | 0 | 0.003 | 0.008 |

***HC – Hydrocortisone acetate; KE-Ketoconazole***

**Table No. 2: Analysis data of Formulations**

| Parameters | HC* | KE* |
|---|---|---|
| **Label claim** | 1% w/v | 2%w/v |
| **% Drug content** | | |
| **Method I** | 98.85 | 100.2 |
| **Method II** | 101.45 | 97.25 |

**Table No. 3: Intermediate Precision studies for Simultaneous Equation and Multicomponent mode methods**

| Parameter | Mean ± %RSD | Mean ± %RSD | Mean ± %RSD | Mean ± %RSD |
|---|---|---|---|---|
| **Drugs** | HC | KE | HC | **KE** |
| **Intra day** | 99.93±0.94 | 104.03±0.53 | 100.61±0.33 | **98.64±0.33** |
| **Inter day** | **98.9±0.70** | **102.33±1.49** | **101.09±0.82** | **97.89±0.76** |

***Denotes average of six estimations. (% Label Claim)***

**Table No. 4: Recovery Studies**

| Drug | Conc of Drug added (µg/mL) | | | % Level | % Recovery | |
|---|---|---|---|---|---|---|
| | Form* | Pure | Total | | Method I | Method II |
| | 5 | 4 | 9 | 80 | 96.77 | **100.21** |
| **HC*** | 5 | 5 | 10 | 100 | 96.66 | **101.32** |
| | 5 | 6 | 11 | 120 | 96.31 | **100.19** |
| | 10 | 8 | 18 | 80 | 100.27 | **98.1** |
| **KE*** | 10 | 10 | 20 | 100 | 100.4 | **97.6** |
| | **10** | **12** | **22** | **120** | **100.31** | **97.2** |

***HC-Hydrocortisone acetate; *KE- Ketoconazole***

## CONCLUSION

As there is no single UV- Spectrophotometric method developed till date for the estimation of Hydrocortisone acetate and Sulphacetamide sodium in the combined dosage forms these two simple, precise and accurate methods have been developed. Thus these methods can be employed for the routine estimation of HC and KE in bulk as well as in the formulation in a shorter period.

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**How to cite this article:**

RM. Naik et al. SIMULTANEOUS ESTIMATION OF HYDROCORTISONE AND KETOCONAZOLE IN TABLET DOSAGE FORM BY UV-SPECTROSCOPY. J Pharm Res 2019;8(5):323-326. **DOI: https://doi.org/10.5281/zenodo.3236709**

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Source of support: Nil**