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Development and Validation of Simultaneous Spectroscopic Method for Tetracycline Hydrochloride and Clotrimazole

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Abstract: A simple and accurate simultaneous method was developed for tetracycline hydrochloride (TH) and clotrimazole with a mixture of solvents. The combination is used to validate the developed UV-spectrophotometric method as per ICH guidelines. The concentration of Tetracycline hydrochloride & clotrimazole in the sample solution was determined by solving the respective simultaneous equations generated by using absorptivity coefficients and absorbance values of TH & Clotrimazole at these wavelengths 361 nm and 259 nm respectively. and both satisfy Beer's law in concentration ranges of 4-20 µg/ml for TH and 10-30 µg/ml for Clotrimazole. The method was effectively used to estimate TH and clotrimazole in Buccal film without the interference of common excipients.

Keywords: Tetracycline hydrochloride, clotrimazole, simultaneous estimation, UV spectroscopy.

I. INTRODUCTION

A combination of tetracycline hydrochloride and clotrimazole has been devised to treat Staphylococcus aureus-induced infective endocarditis. Antibiotic resistance has developed in Staphylococcus aureus. The S. aureus major facilitator superfamily (MFS) efflux pump Tet causes tetracycline resistance (K). Whereas biofilm hinders the diffusion of antibiotics. None of the compounds that are currently known have had efflux pump inhibitors (EPIs) licensed for use in clinical settings. We discovered clotrimazole, which is inhibitor of fungus-produced ergosterol production, with possible EPI-like potential and fractional inhibitory concentrations, indicating synergism. The literature search revealed that, although a promising combination, there is no method available for simultaneous estimation of TH & Clotrimazole. Therefore, developing a UV spectrophotometric method for the simultaneous estimation of TH and clotrimazole is challenging. Therefore, the aim of the present work was to develop & validate a UV spectroscopic method for simultaneous estimation of TH and Clotrimazole.

Tetracycline hydrochloride, is chemically (4S,4aS,5aS,6S,12aS)-4- Dimethylamino- 3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo- 1,4,4a,5,5a, 6,11,

12a-octahydrotetracene-2- carboxamide monohydrochloride, as shown in fig.1^[1]

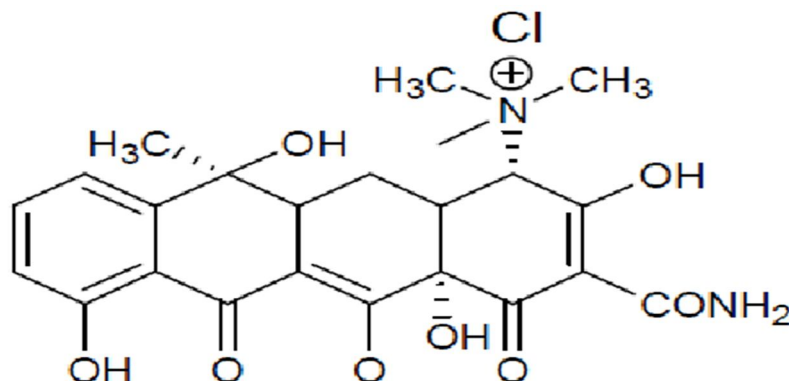


Fig 1.

Whereas, clotrimazole is chemically 1-[(2-Chlorophenyl) (diphenyl)methyl]-1H-imidazole as shown in the fig. 2

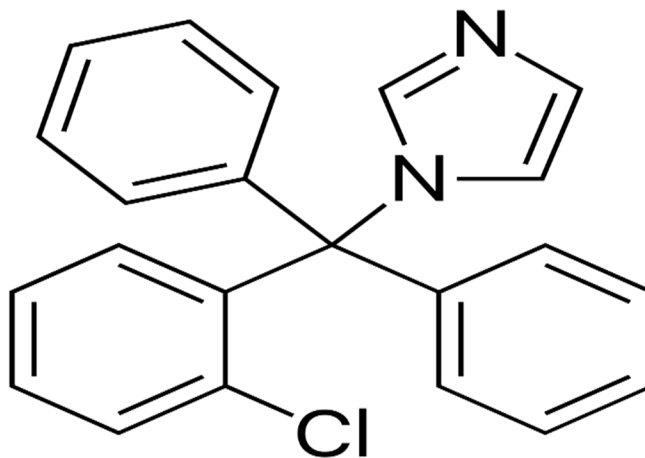


Fig 2.

Over the range of 200-400 nm, a UV-spectrophotometer UV-1800 (Shimadzu, Japan) with a spectral bandwidth of 2 nm and 10 mm matched quartz cells was utilised to create an analytical procedure.

II. MATERIAL AND METHOD

A. Chemical and Instrument

The reference standard Clotrimazole (99.4%) was received as a gift sample from halcyon sewri west, Mumbai-400015, and tetracycline hydrochloride (99%) was received as a gift sample from Medley, Andheri, Mumbai-400069. All solvents used for analysis were of analytical grade and were procured from Vishal Chem. India.

Over the range of 200-400 nm, a UV-spectrophotometer UV-1800 (Shimadzu, Japan) with a spectral bandwidth of 2 nm and 10 mm matched quartz cells was utilised to create an analytical procedure.

B. Development of the Method

During the development of the method, the spectrum of each drug was measured in different solvents. This was done to enable the selection of the solvent system and concentration range. Based on the spectrum, the solvent selected was methanol and water for both drugs. The method was developed and validated as per the procedures mentioned.

C. Preparation of Standard Stock Solution

Accurately weighed clotrimazole (50 mg) and tetracycline hydrochloride (TH) (50 mg) were transferred to a 50 mL volumetric flask, dissolved, and diluted to the mark with methanol to obtain a standard solution having a concentration of tetracycline and clotrimazole (1000 ppm). Once more, 10 ml of TH and 10 ml of clotrimazole were withdrawn respectively, and diluted to 100 ml each with methanol in a volumetric flask of 100 ml (100 ppm).

D. Calibration curve of Tetracycline Hydrochloride and Clotrimazole

A series of calibrated 10mL volumetric flasks were taken and appropriate aliquots of stock solution of TH and clotrimazole were withdrawn and diluted up to 10mL with water^[2]. Different dilutions were made from this stock solution, ranging from 4 µg/ml to 20 µg/ml for TH. The absorbance was measured at absorption maxima 361 nm for TH against the reagent blank prepared in a similar manner without TH i.e., water. The same procedure was applied for clotrimazole stock solution solutions having concentrations of 10, 15,20,25,30 µg/mL) and absorbance was measured at 259nm, against a reagent blank prepared in a similar manner without clotrimazole.

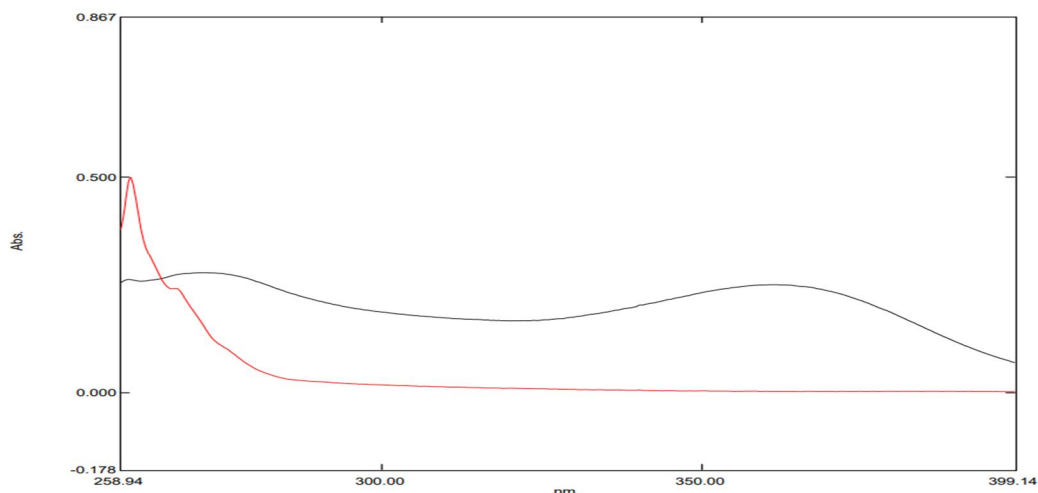


Fig 3. UV Spectrum of Tetracycline hydrochloride and Clotrimazole

E. Method Validation

1) Linearity

The calibration curve was created by comparing the absorbance of different concentrations of TH to a blank of methanol. The graph was made in order to investigate the linear relationship between absorbance and concentration from this stock solution, ranging from 4 µg/ml to 20 µg/ml.

Sr.no.	Concentration(µg/ml)	Absorbance
1.	BLANK	0.00
2.	4	0.149
3.	8	0.278
4.	12	0.422
5.	16	0.572
6.	20	0.681

Table no 1: Standard Calibration for TH at 361 nm

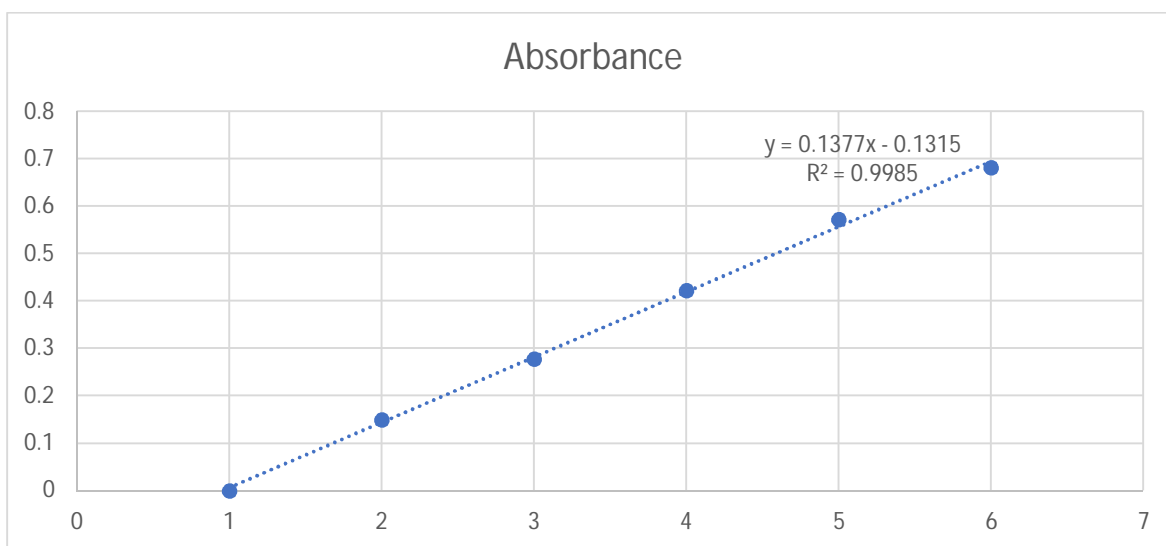


Fig 4. Standard Calibration curve of TH

Figure no 4: TH shows the linearity range of 4 – 20 µg/ml having the line equation of $y=0.1377x$ and regression value of 0.9985. The regression value indicates that the point lies near the line approaches 1.

Sr.no.	Concentration(µg/ml)	Absorbance
1.	BLANK	0.00
2.	10	0.169
3.	15	0.344
4.	20	0.508
5.	25	0.695
6.	30	0.870

Table no 2: Standard Calibration for Clotrimazole at 259 nm

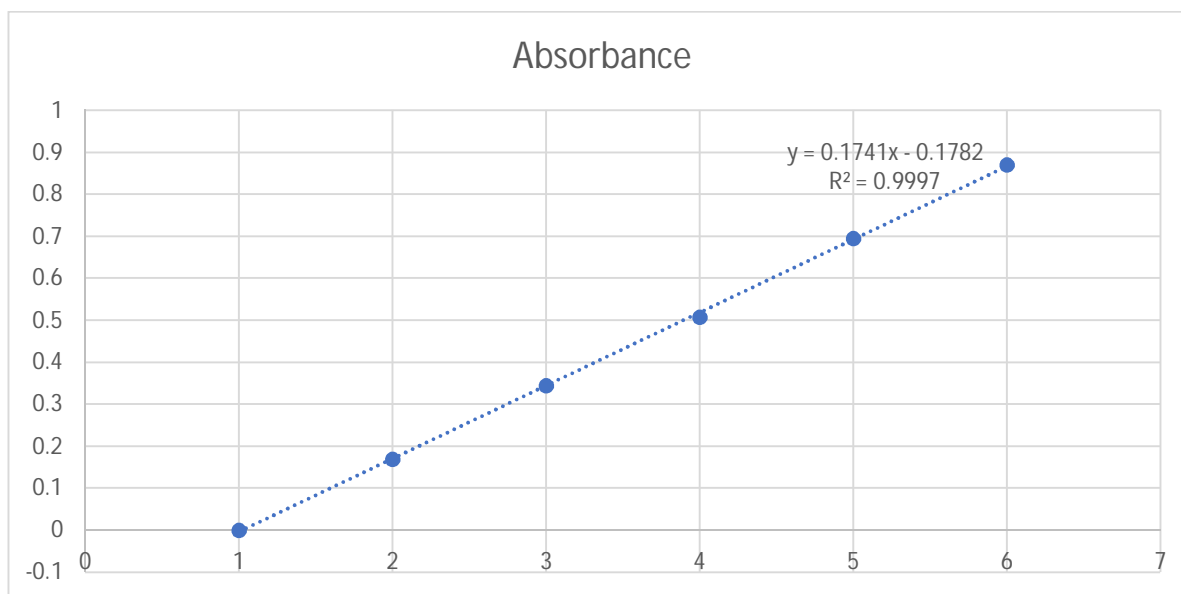


Fig 5. Calibration curve of clotrimazole

Figure no 7: clotrimazole shows the linearity range of 10 – 30 µg/ml having the line equation of $y=0.1741x$ and regression value of 0.9997. The regression value indicates that the point lies near the line approach 1.

2) Molar absorptivity

Molar absorptivity (ϵ) it is a measurement of how strongly a chemical species absorb light at a given wavelength. It is an intrinsic property of the species. The actual absorbance (A) of a sample is dependent on the path length (l) and the concentration (c) and is given as-

$$[A = \epsilon c l]$$

The unit used to describe the molar absorptivity is L/mol/cm.

Calculation of molar absorptivity: -

$$\text{Molar absorptivity } (\epsilon) = \text{Absorbance/molar concentration}$$

Calculation of percent absorptivity: -

$$\% \text{ Absorptivity} = \text{Absorbance/concentration in mg/ml}$$

Wavelength (nm)	Molar absorptivity of (ϵ) TH	Molar absorptivity (ϵ)of clotrimazole
361	0.0358133	0.000796
259	0.001568	0.0245733

Table no 3: Molar absorptivity of TH and clotrimazole

3) Precision

Intraday Precision

Solutions containing 4-20 µg/mL of TH and 10-15 µg/mL of clotrimazole at low, middle, and high concentration were analyzed 3 times on the same day and % RSD was calculated.

Interday Precision

Solutions containing 4-20 µg/mL of TH and 10-15 µg/mL of clotrimazole at low, middle, and high concentration were analyzed 3 times on on 3 subsequent days and % RSD was calculated.

4) Accuracy

The accuracy of the method was determined by calculating the recovery of TH & Clotrimazole by the standard addition method. Known amounts of standard solutions of TH & Clotrimazole were added at 80, 100, and 120% level to sample solutions of TH & Clotrimazole (20 µg/ml for TH and 10 µg/ml for clotrimazole). The experiment was performed in triplicate.

5) Limit of Detection and Limit of Quantitation

The LOD and LOQ were determined based on the standard deviation of the response and the slope.

$$LOD = 3.3 \sigma \div S \text{ and } LOQ = 10 \sigma \div S$$

Where,

σ = standard deviation of the response

S = slope of the calibration curve (of the analyte)

6) Assay

Buccal film of 2x2 equivalent to 20mg of TH and 10mg of clotrimazole were transferred into a 100 ml volumetric flask. phosphate buffer (pH 6.8) was added to 100 ml mark the solution was shaken well and then filtered off using whatman filter paper the concentration of the above solution are TH (200 µg/ml) and clotrimazole (100 µg/ml) 1 ml of above solution was pipette out and make up with phosphate buffer in 10 ml volumetric flask (1st dilution) 1 ml of was pipette out and make up with phosphate buffer in 10 ml volumetric flask (2nd dilution) the absorbance of 2nd dilution solution was measured at 361 nm and 259 nm

III. RESULT AND DISCUSSION

A. Determination of Solubility

Solvent	Tetracycline hydrochloride	Clotrimazole
Water	Soluble	Insoluble
Methanol	Soluble	Soluble
Ethanol	Slightly soluble	Slightly soluble
DMSO	Soluble	Soluble

Table no.4

Considering the solubilities of both the drugs, the stock solutions were prepared in Methanol, followed by dilution in Water.

B. Linearity

The linearity of proposed method was determined from the calibration curve data of both the drugs that is TH and clotrimazole. TH shows linear response between 4 - 20 µg/ml and clotrimazole shows linear response between 10 – 30 µg/ml. Acceptance criteria usually involve a Goodness of Fit test. A high correlation coefficient (r²) of 0.99 is often used as criterion of linearity. However, this is not sufficient to prove that a linear relationship exists, and a method with a coefficient of determination of less than 0.99 may still fit for process.

Slope = 0.1377x for TH

Slope = 0.1741x for clotrimazole

(r²) for TH = 0.998

(r²) for clotrimazole = 0.999

C. Precision

Intraday Precision:

Solutions containing 4-20 µg/ml of TH and 10-30 µg/mL of clotrimazole were analyzed 3 times on the same day and % RSD was calculated.

Interday Precision

Solutions containing 4-20 µg/ml of TH and 10-30 µg/mL of clotrimazole were analyzed 3 times on three consecutive days and % RSD was calculated.

D. Limit of Detection

The lowest concentration of analyte in a sample that can be detected but not necessarily quantified using a certain method under the proper experimental conditions is known as the limit of detection. This limit is expressed in terms of analyte concentration in the sample.

$$\% \text{ RSD} = \sigma \times \text{mean} / 100, \text{ CV} = \sigma / \text{mean}$$

Limit of detection of TH

$$[\text{LOD} = 3.3 \times \sigma / s]$$

Where, Standard = 0.003745, Slope (s) = 0.1377, $\text{LOD} = 3.3 \times 0.003745 / 0.1377 = 0.089758$ µg/ml.

LOD of Clotrimazole

Where, Standard = 0.004347, Slope (s) = 0.1741, $\text{LOD} = 3.3 \times 0.004347 / 0.1741 = 0.082403$ µg/ml.

E. Limit of Quantitation

The Limit of quantitation is the lowest concentration of analyte in a sample which can quantitatively determine with suitable accuracy and precision under the stated operational condition of the method. Limit of quantitation can vary with the type of method employed and the nature of the sample. It is based on the standard deviation of the response and the slope.

Limit of quantitation of TH

$$[\text{LOQ} = 10 \times \sigma / s]$$

Where, Standard = 0.003745, Slope (s) = 0.1377, $\text{LOQ} = 10 \times 0.003745 / 0.1377 = 0.2719$ µg/ml.

Limit of quantitation of Clotrimazole

Where, Standard = 0.004347, Slope (s) = 0.1741, $\text{LOQ} = 10 \times 0.004347 / 0.1741 = 0.2497$ µg/ml.

F. Accuracy (Recovery Test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to suspension. The recovery was performed at three levels, 80, 100 and 120% of Artemether and Lumefantrine standard concentration. The recovery samples were prepared. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated using formula:

$$\% \text{ recovery} = \frac{\text{Observed amount of compound in sample}}{\text{Amount of all compound present in sample}} \times 100$$

Conc. Of test solution	Absorbance of test solution	Conc. of standard solution	Absorbance of standard solution	Absorbance of spiked solution	% Recovery	% Mean recovery
20	0.504	18	0.281	0.775 0.779 0.780	96.44 97.86 98.22	97.5066667
20	0.502	20	0.380	0.869 0.870 0.873	96.57 96.84 97.63	97.01333
20	0.502	22	0.509	0.988 0.989 0.989	95.481 95.67 95.677	95.60933

Table no.5 Accuracy of Tetracycline hydrochloride:

Accuracy				% Recovery		
80%				97.5066667		
100%				97.01333		
120%				95.60933		
Conc. Of test solution	Absorbance of test solution	Conc. of standard solution	Absorbance of standard solution	Absorbance of spiked solution	% Recovery	% Mean recovery
10	0.178	8	0.132	0.311 0.309 0.310	100.75 0.99.24 100	100.375
10	0.178	10	0.389	0.570 0.567 0.568	100.77 100 100.25	100.34
10	0.178	12	0.509	0.699 0.702 0.701	100.23 102.94 102.75	101.973333

Table no. 6 Accuracy of Clotrimazole:

Accuracy	% Recovery
80%	100.375
100%	100.34
120%	101.973333

From the result of recovery studies, it was found that the percent recovery values of pure drug from the analyzed solution were between 95 to 102 %, which indicates that the method is accurate and reveals that commonly used excipients present in pharmaceutical formulation did not interfere in this method. The accuracy of the method was validated by recovery studied and was found to be significant and under specification limit (ie within the acceptable range 95-120%)

Parameter	Tetracycline hydrochloride	Clotrimazole
Wavelength range (nm)	361nm	259nm
Linearity & Range (µg/mL)	4-20 µg/ml	10-30 µg/ml
Regression equation (y = mx + c)	y = 0.1377x - 0.1315	y = 0.1741x - 0.1782
Slope	0.1377	0.1741
Correlation coefficient (r)	0.998	0.999
Precision (%RSD)		
1a. Intraday Precision	0.221-1.0228	0.229-2.094
1b. Interday Precision	0.1443-1.54303	0.196-1.0371
Accuracy (% Recovery)	95.6-97.50	100.34-101.97
LOD	0.089758	0.082403
LOQ	0.271992	0.249707

The current paper discusses the use of UV spectrophotometry to estimate and validate TH and clotrimazole in buccal dose form. Following a review of the literature, it was discovered that TH and clotrimazole can be estimated separately using several HPLC, other analytical methods, and that only a few individual methods for estimation of artemether and lumefantrine by UV spectrophotometry are available. Since there are relatively few methods for estimation of TH and clotrimazole in combined dosage form, it was felt that developing a specific method for their combination was necessary. Simultaneous spectroscopic estimation of TH and clotrimazole was performed using the simultaneous equation approach, in which separate forms of TH and clotrimazole were estimated using methanol and distilled water solvent and a calibration curve. The absorption maxima for Tetracycline hydrochloride are at 361 and for clotrimazole are at 259. Beer's rule is followed across a concentration range of 4–20 µg/ml for TH, and 10–30 µg/ml. for Clotrimazole. The devised approach has been validated in accordance with ICH criteria from 1996

IV. CONCLUSION

The quantitative determination of tetracycline hydrochloride and clotrimazole using UV spectroscopy was done quickly, cheaply, linearly, repeatedly, specifically, and economically. After the technique was validated, it was determined that all the method validation parameters were satisfactory. Regular analysis of samples of marketed tetracycline hydrochloride and clotrimazole as well as in-process quality control can be done using the described method.

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