SIMULTANEOUS ESTIMATION OF CHLORHEXIDINE AND TRIAMCINOLONE BY HPLC

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ABSTRACT
A Reverse phase high performance liquid chromatographic method is developed for the simultaneous determination of Chlorhexidine Hydrochloride and Triamcinolone in Lozenges (in house) is reported. Reverse phase chromatography was developed using Waters symmetry C18 column (250 X4.6 mm) with 5 µm particle size monitored at 254nm with a mobile phase MeOH: Water: Glacial acetic acid (75:25:10) is used with ion pair reagent Octane-1-sulfonic acid sodium salt (0.2 gm). The method is validated with the range of 25-125 µg/ml and 5-25 µg/ml and correlation coefficients were found to be 0.997 and 0.999 for Chlorhexidine Hydrochloride and Triamcinolone respectively. Recovery studies showed good results 98.93% for Chlorhexidine Hydrochloride and 99.95% for Triamcinolone. Coefficients of variation for precision ranging from 0.14% to 1.32% and 0.15% to 0.67% for Chlorhexidine Hydrochloride and Triamcinolone respectively. The chromatographic method provides rapid and precise method for separation. The method is accurate, specific and reliable, and can be successfully used for the quality control on bulk product and pharmaceutical dosage form.

Keywords: Chlorhexidine hydrochloride (CHH), Triamcinolone (TRI), Ion pair chromatography, Octane-1-sulfonic acid sodium salt.

INTRODUCTION
Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC and HPTLC etc. These methods are accurate and precise with good reproducibility. Chlorhexidine is a chemical antiseptic. It is effective on both Gram-positive and Gram-negative bacteria. It has both bactericidal and bacteriostatic mechanisms of action, the mechanism of action being membrane disruption, not ATPase inactivation as previously thought. It is also useful against fungi and enveloped viruses, though this has not been extensively investigated. Chlorhexidine is harmful in high concentrations, but is used safely in low concentrations in many products, such as mouthwash and contact lens solutions. By ionization it produces positive ions.
Triamcinolone is a long-acting synthetic corticosteroid given orally, by injection, inhalation, or as a topical ointment or cream. Early anti-inflammatory effects include the inhibition of macrophage and leukocyte movement and activity in the inflamed area by reversing vascular dilation and permeability. Later inflammatory processes such as capillary production, collagen deposition, keloid (scar) formation also are inhibited by corticosteroids. Clinically, these actions correspond to decreased edema, erythema, pruritus, plaque formation and scaling of the affected skin. Triamcinolone is used to treat several different medical conditions, such as eczema, psoriasis, arthritis, allergies, ulcerative colitis, lupus, sympathetic ophthalmia, temporal arteritis, uveitis, and ocular inflammation, visualization during vitrectomy and the prevention of asthma attacks.\textsuperscript{[4]}

![Figure 1](a) Chlorhexidine Hydrochloride and (b) Triamcinolone

Chlorhexidine is weekly retained by reverse phase chromatography so ion pair reagent (Octane-1-sulfonic acid sodium salt) is used for the separation of the combination.

**Ion pair Chromatography**\textsuperscript{[5]}:

The stationary phases used are completely polar reversed phase materials such as are used in distribution chromatography. A so-called ion pair regent is added to the eluents; this consists of anionic or cationic surfactants such as tetra-alkyl ammonium salts or n-alkylsulfonic acids. Together with the oppositely charged analyte ions the ion pair reagents form an uncharged ion pair, which can be retarded at the stationary phase by hydrophobic interactions. Separation is possible because of the formation constants of the
ion pairs and their different degrees of adsorption. The different columns used are octa
decyl silane (ODS) or C18, C8, C4, etc., (in the order of increasing polarity of the
stationary phase).

**Ion Pairing Reagents:**
- Ion-pairing agents are ionic compounds that contain a hydrocarbon chain that
  imparts certain hydrophobicity so that the ion pair can be retained on a reversed-
  phase column.
- Ion Pairing agents are added at concentrations of 0.05 to 0.2M.
- Ion pair reagents are available in two series
  - S – series (Sodium salt of alkane sulfonates)
  - Q – series (Quaternary alkyl triethyl amines)

Literature assessment [6-14] showed that various analytical methods have been
reported for the estimation of Chlorhexidine and Triamcinolone individually and with
other drugs combination by UV, HPLC, GC-MS and LC-MS in pharmaceutical dosage
forms and biological fluids. There is no work done on this combination and this
combination is very useful in oral infection as well as throat infection. The present work
aims at developing newer RP-HPLC method that are simple, accurate, rapid, precise,
sensitive and reliable for the estimation of Chlorhexidine and Triamcinolone
combination in bulk drug.

**MATERIALS AND METHODS**

**MATERIALS**

Reagent and Chemicals
Chlorhexidine HCl and Triamcinolone Working standard grade was supplied by ICPA
Health Products Ltd. (Ankleshwar, India). HPLC Grade Methanol, HPLC grade Glacial
Acetic Acid, HPLC Grade Water (Milli-Q), Octane-1-sulfonic Acid Sodium salt (For ion
pair Chromatography) is used for the work. All chemicals and reagents used were of
HPLC grade mfg. by Merck Laboratories.

Instruments and equipments
HPLC LC-2010C HT Shimadzu with LC Solution Waters symmetry C18, 5 (250 X4.6
mm, 5 µm particle size), Perkin Elmer lambda 25 UV/Vis Double beam
spectrophotometer and Calibrated glassware’s were used for the study. Ultipor N66 Nylon 6.6 Membrane 0.2 μm filter is used for HPLC.

METHODOLOGY

Preparation of working stock solution of CHH and TRI:

CHH solution: (500 μg/ml)
An accurately weighed quantity of 100.0 mg Chlorhexidine Hydrochloride was transferred in 100.0 ml volumetric flask, dissolved with 25 ml of HPLC mobile phase and volume was made up to the mark with the same. From the above solution of CHH take 50 ml of solution in 100 ml of volumetric flask and make up the volume up to the mark with HPLC mobile phase.

TRI solution: (100 μg/ml)
An accurately weighed quantity of 50.0 mg Triamcinolone was transferred in 100.0 ml volumetric flask, dissolved with 25 ml of HPLC mobile phase and volume was made up to the mark with the same. From the above solution of TRI take 20 ml of solution in 100 ml of volumetric flask and make up the volume up to the mark with HPLC mobile phase.

Selection of detection wavelength:

Accurately weighed 50 mg of CHH and 10 mg of TRI were taken and transferred into 100ml volumetric flask individually. Then mobile phase is added to make up volume up to the mark. The solution was sonicate for 15 min and filtered through Ultipore N66 Nylon 6, 6 membrane filter paper. The filtrates (1.0ml) of both were further diluted to 10ml with mobile phase. Final concentration contains 50 μg/ml of CHH and 10 μg /ml TRI and spectrum were recorded at UV- Spectrophotometer.
Preparation of Calibration curve for CHH and TRI:
Aliquots ranging from 0.5 ml to 2.5 ml were taken from working stock solution of CHH and TRI in 10 ml volumetric flask and diluted to volume with mobile phase to give final concentrations of 25, 50, 75, 100, 125 µg/ml and 5, 10, 15, 20, 25 µg/ml of CHH and TRI respectively. Injections of 20 µl were made for each concentration and chromatogram was obtained under the condition described as above at 254 nm wavelength. Calibration graph was constructed by plotting peak area versus concentration of each drug and the regression equation was calculated.

Preparation of sample solution from Formulation:
Ten lozenges were accurately weighed and finely powdered. The powder equivalent to 50 mg of CHH was accurately weighed and transferred into 100ml volumetric flask. Then mobile phase is added to make up volume up to the mark. The solution was sonicated for 15 min and filtered through Ultipore N66 Nylon 6, 6 membrane filter paper. The filtrate (1.0ml) was further diluted to 10ml with mobile phase. Final test solution contains 50 µg of CHH and 10 µg of TRI per ml of final solution.

Validation: Validation of this method was done as per ICH guidelines. [15,16]

RESULT AND DISCUSSION
Final Chromatographic condition:
The mixed standard solution containing 50 g/ml of CHH and 10 g/ml of TRI was analyzed using mobile phase of varied ratios illustrated in table 7.1. The chromatograms were recorded. A composition of MeOH: Water: Glacial acetic acid (75:25:10) [OSA 0.2 gm] showed good resolution with acceptable retention time.

A satisfactory separation for the two drugs were obtained with mobile phase consisting of MeOH: Water: Glacial acetic acid (75:25:10) [OSA 0.2 gm] at ambient temperature with flow rate of 1.0 ml/min. Waters symmetry C18, 5 (250 X 4.6 mm, 5 µm particle size) column showed better resolution. Quantization was achieved with UV detection at 254 nm based on peak area.

**Figure 3**

Chromatogram of CHH and TRI in MeOH: Water: Glacial acetic acid (75:25:10) [OSA 0.2 gm]

**Method Validation:**

**Specificity:**
Linearity and Range
Chlorhexidine hydrochloride and Triamcinolone were found to linear in the range of 25µg/ml-125 µg/ml and 5 µg/ml-25 µg/ml respectively.
Table 1: Regression Analysis of CHH and TRI

<table>
<thead>
<tr>
<th>Regression Analysis</th>
<th>CHH</th>
<th>TRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression line equation</td>
<td>Y=105585x-24574</td>
<td>Y=41714x+603.5</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.998</td>
<td>0.999</td>
</tr>
<tr>
<td>Slope</td>
<td>105585</td>
<td>41714</td>
</tr>
<tr>
<td>Intercept</td>
<td>24574</td>
<td>603.5</td>
</tr>
</tbody>
</table>

**Precision**

% RSD for Repeatability, Interday and Intraday were found to be 0.32 and 0.14, 0.19-0.60 and 0.23-0.87, 0.39-0.67 and 0.24-1.32 for CHH and TRI respectively.

**Accuracy**

Accuracies for working standard of CHH and TRI at 50%, 100% and 150% levels were found to be 99.44% and 100.53%, 99.20% and 99.15% and 99.78% and 99.52% respectively.

**System suitability:**

![TRI Calibration Curve](image-url)
### Table 2: Values of System Suitability Parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>CHH</th>
<th>TRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Peak area</td>
<td>5247643</td>
<td>418275</td>
</tr>
<tr>
<td>2.</td>
<td>No. of theoretical plates</td>
<td>7893.351</td>
<td>5796.683</td>
</tr>
<tr>
<td>3.</td>
<td>Retention time (min)</td>
<td>9.245</td>
<td>4.249</td>
</tr>
<tr>
<td>4.</td>
<td>Tailing Factor</td>
<td>1.080</td>
<td>1.213</td>
</tr>
<tr>
<td>5.</td>
<td>Resolution</td>
<td>16.499</td>
<td></td>
</tr>
</tbody>
</table>

**Summary of Validation Parameters:**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CHH</th>
<th>TRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range (µg/ml)</td>
<td>25-125</td>
<td>5-25</td>
</tr>
<tr>
<td>Regression line equation</td>
<td>Y=105585x-24574</td>
<td>Y=41714x+603.5</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.998</td>
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<td>41714</td>
</tr>
<tr>
<td>Intercept</td>
<td>24574</td>
<td>603.5</td>
</tr>
<tr>
<td>Repeatability (%RSD, n=10)</td>
<td>0.32</td>
<td>0.14</td>
</tr>
<tr>
<td>Interday (%RSD) (n = 3)</td>
<td>0.19-0.60</td>
<td>0.23-0.87</td>
</tr>
<tr>
<td>Intraday (%RSD) (n = 3)</td>
<td>0.39-0.67</td>
<td>0.24-1.32</td>
</tr>
<tr>
<td>%Recovery (accuracy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 1</td>
<td>99.44</td>
<td>100.53</td>
</tr>
<tr>
<td>Level 2</td>
<td>99.20</td>
<td>99.15</td>
</tr>
<tr>
<td>Level 3</td>
<td>99.78</td>
<td>99.52</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>5.0904</td>
<td>0.278</td>
</tr>
<tr>
<td>LOQ(µg/ml)</td>
<td>15.425</td>
<td>0.842</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
<td>Specific</td>
</tr>
<tr>
<td>% assay (n=3)</td>
<td>99.96</td>
<td>100.40</td>
</tr>
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</table>

www.pharmasm.com  IC Value – 4.01  2858
CONCLUSION
The value of %RSD for intraday and inter-day precision was found less than 2. This value confirms that method is precise. The value of %recovery greater than 98% for this method shows that the method is accurate. The values of %recovery for analysis of formulations are found within 99-101%.

The proposed reverse phase HPLC method is found to be specific, accurate and precise. The proposed HPLC method can be successfully utilized for the simultaneous estimation of CHH and TRI in the combined dosage form without any prior separation of individual drugs.

ACKNOWLEDGEMENT
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