Compatibility and stability studies of erythromycin Estolate and Piperine mixture

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ABSTRACT

Erythromycin and its derivatives are used to treat variety of Bacterial infections in Humans. Piperine, an alkaloid from Piper species is reported to enhance oral bioavailability of co-administered drugs. The present study was aimed to include to Piperine (bioenhancer) as a formulation additive in oral formulations of Erythromycin estolate. So, a Physical mixture of Erythromycin estolate and Piperine (1:1) was tested for their compatibility by DSC, TLC and FT-IR techniques. Stability of the mixture was assessed by the use of ICH guidelines. The above studies have proved that Piperine can be used as a formulation additive (bioenhancer), in oral formulations of Erythromycin estolate.

Keywords: Erythromycin, Piperine, Bioenhancer. Compatibility studies, Stability studies

INTRODUCTION

Piperine (1-piperoyl piperidine) is a major component of the Piper species. This species has been used widely as spices and in various systems of Indian medicine10. Piperine has been shown to enhance the bioavailability of drugs like Vascicine, Curcumin, Barbirturate, Oxypentylbutazone, Propranolol, Theophylline and Erythromycin.11,12 in animal experiments. Erythromycin is extensively used in various bacterial infections10. The present study was aimed to develop a new concept of inclusion of bioenhancer as a formulation additive by conducting compatibility and stability studies of Erythromycin and Piperine (1:1) mixture.

MATERIALS AND METHODS

Piperine sample was received from M/s Sami labs, Bangalore. Erythromycin estolate was purchased from M/s. MMC Laboratories, Chennai and the sample was found to comply with specifications as per Indian Pharmacopoeia9. The test microorganism, Micrococcus luteus (ATCC 10240) was obtained from National Chemical Laboratories (NCL), Pune. The Culture and Assay Media were supplied by M/S Hi-Media Ltd., Mumbai. Differential Scanning Calorimeter (Model DSC 60) was purchased from M/S Shimadzu, Japan. The Fourier Transform-Infra Red Spectrometer (Model: Spectrum One) was purchased from M/S Perkin Elmer, USA. Compatibility studies of Ampicillin Trihydrate Piperine physical mixture (1:1) was carried out using Differential Scanning Calorimetry (DSC), Thin Layer Chromatography (TLC) and Fourier Transform Infra Red (FT-IR) Spectroscopy. The same mixture was used for stability studies and the studies were carried out in Stability Study Chamber (Model: Ketan), manufactured by M/s. Shivani Scientific Industries (P) Ltd., Mumbai. The Potency of Ampicillin (for stability studies) was determined microbiologically (Filter paper disc – diffusion assay) using Bacillus subtilis (ATCC 6633) as test organism9,10.

Experimental Design

a. Compatibility studies

Compatibility studies are an important step in pre formulation studies for developing a dosage form. The Erythromycin estolate-Piperine physical mixture (1:1) was stored in High Density Poly ethene (HDPE) containers sealed with Teflon. The mixture was subjected to Differential Scanning Calorimetry (DSC) studies [scanning temperature range 35°C – 275°C with (10°C/min. raise)], Thin Layer Chromatography (TLC) studies for Erythromycin estolate (9) and Fourier Transform - Infra Red (FT-IR) studies (scanning between 400 cm

1

and 4000 cm

1

) at zero time and one month after storing at 50°C in an oven11,12.

b. Stability studies

Stability studies are also an important part of pre formulation studies. The stability of the drug substance depends upon the container and conditions in which it is stored and other excipients present with the drug. Since the storage temperature for Erythromycin estolate, as recommended by many Pharmacopoeias is around 25°C, the stability studies for Erythromycin estolate-Piperine physical mixture (1:1), was carried out as per ICH guidelines for General cases of drugs9,12. For this study Erythromycin estolate was hydrolysed to Erythromycin13 and then mixed with Piperine in the said ratio. The mixture was encapsulated in hard gelatin capsule shells, the capsules were then transferred to High Density Poly Ethylene (HDPE) bottles. The mouth of the bottles were then covered with aluminium foil and then closed with a closure. The study was carried out as accelerated study (for 6 months) by storing at 40°C ± 2°C, 75 ± 5% RH (sampling at 0,1,2,3 months) and as long term study (for 1 year) by storing at 25°C ± 2°C, 60±5%RH sampling was done at 0, 3, 6, 9, 12 months.

Sampling:
The quantity of mixture equivalent to 50mg of Erythromycin was sampled and Piperine was separated from the mixture by addition of 5ml of 0.1N acetic acid (in which Piperine is soluble), the resultant solution was filtered by using Whatman filter paper (mean pore size 45µm) to separate Erythromycin. After separation, the resulting solution was suitably diluted with sterile phosphate buffer (pH 8) to get a concentration of 5µg/ml of Erythromycin.

The results for long term stability studies of the mixtures are given in Table 2 and the results for Accelerated stability studies are given in Table 3. The degradation rate constants were calculated from stability profiles (% potency retained vs. time) and the results are given in Table 4.

RESULTS AND DISCUSSION

A. Compatibility Studies

i. DSC Studies

From the above thermograms, it can be observed that there is no endothermic peak corresponding to the melting range of Erythromycin estolate, which is 135°C-140°C10. The endothermic peak obtained with Erythromycin estolate and Piperine mixture (fresh and stored) corresponds to melting range of Piperine (128º to 129°C)11. So it can be assumed that Erythromycin estolate and Piperine do not interact immediately upon mixing and even after storage of mixture (Fig 1 to 2).

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The results of the TLC studies are given in Table – 1 and the results of DSC and Fourier Transform- Infra Red (FT-IR) studies are presented as figures. (For DSC studies Fig.:1 & 2 and for FT-IR studies Fig.3 to 6).

REFERENCES


Figure 1: DSC thermograms of Erythromycin estolate-Piperine system. (A) Erythromycin estolate, (B) Erythromycin estolate-Piperine fresh mixture and (C) Stored mixture.

Figure 2: DSC Thermogram of Piperine.

ii. TLC studies
The results of TLC studies (Table-1) show no change in the Rf values of Erythromycin estolate in combination with Piperine in fresh as well as in stored conditions. This indicates that no interaction takes place between Erythromycin estolate and Piperine in fresh as well as stored conditions.

Table 1 Results of TLC studies

<table>
<thead>
<tr>
<th>Combination</th>
<th>RF Value (mean±S.D)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin estolate</td>
<td>0.83 ± 0.20</td>
</tr>
<tr>
<td>Erythromycin estolate + Piperine (Fresh Mixture)</td>
<td>0.83 ± 0.21</td>
</tr>
<tr>
<td>Erythromycin estolate + Piperine (Stored Mixture)</td>
<td>0.83 ± 0.12</td>
</tr>
</tbody>
</table>

*ns=6, P < 0.01

iii. FT-IR studies
The Fourier Transform-Infra Red spectra (Fig.3 to 6) of mixture of Erythromycin estolate and Piperine (fresh as well as stored) are given below and they show the characteristic peaks (with wave numbers) of both Erythromycin estolate and Piperine. The spectra show the characteristics of a Physical mixture of Erythromycin estolate and Piperine in fresh as well as in stored conditions. This is an indication that there is no interaction between Erythromycin estolate and Piperine immediately and upon storage.

b. Stability studies
The results of stability studies (presented as tables 2, 3 & 4) show that the mixtures of Erythromycin and Piperine are stable during long term study (storage at 25°C ± 2°C, 60 ± 5% RH) and even at accelerated conditions (storage at 40°C ± 2°C, 75±5%RH). The degradation (indicated by decrease in % potency of antibiotic) followed zero order kinetics and the degree of degradation was less than 5%. But there was no change in the physical properties such as colour, odour and taste of the mixture. The pungency of Piperine was retained in both long term and accelerated stability studies.
Table 2: Results of long term stability studies for erythromycin estolate & piperine mixture

<table>
<thead>
<tr>
<th>Testing (Months)</th>
<th>Colour of the mixture</th>
<th>Odour &amp; Taste of the mixture</th>
<th>% Potency retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>colourless</td>
<td>Slightly Pungent</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>colourless</td>
<td>Slightly Pungent</td>
<td>99.4±0.85</td>
</tr>
<tr>
<td>6</td>
<td>colourless</td>
<td>Slightly Pungent</td>
<td>98.7±0.68</td>
</tr>
<tr>
<td>9</td>
<td>colourless</td>
<td>Slightly Pungent</td>
<td>98.0±0.92</td>
</tr>
<tr>
<td>12</td>
<td>colourless</td>
<td>Slightly Pungent</td>
<td>97.2±0.52</td>
</tr>
</tbody>
</table>

(*n=3, mean ± S.D, P<0.05)

Table 3: Results of accelerated stability studies for erythromycin estolate & piperine mixture

<table>
<thead>
<tr>
<th>Testing (Months)</th>
<th>Colour of the mixture</th>
<th>Odour &amp; Taste of the mixture</th>
<th>% Potency retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>colourless</td>
<td>Slightly Pungent</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>colourless</td>
<td>Slightly Pungent</td>
<td>99.5±0.80</td>
</tr>
<tr>
<td>2</td>
<td>colourless</td>
<td>Slightly Pungent</td>
<td>99.1±0.65</td>
</tr>
<tr>
<td>3</td>
<td>colourless</td>
<td>Slightly Pungent</td>
<td>98.7±0.86</td>
</tr>
<tr>
<td>6</td>
<td>colourless</td>
<td>Slightly Pungent</td>
<td>97.5±0.94</td>
</tr>
</tbody>
</table>

(*n=3, mean ± S.D, P<0.05)
Table 4: Zero order degradation rate constants for Erythromycin estolate & Piperine mixture.

<table>
<thead>
<tr>
<th>Name of the mixture</th>
<th>Zero order rate constant/month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40°C ± 2°C, 75 ± 5% RH</td>
</tr>
<tr>
<td></td>
<td>25°C ± 2°C, 60 ± 5% RH</td>
</tr>
<tr>
<td>(Accelerated Condition)</td>
<td>(Long Term)</td>
</tr>
<tr>
<td>Erythromycin estolate &amp; Piperine</td>
<td>0.4 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>0.2 ± 0.32</td>
</tr>
</tbody>
</table>

(*n=3, mean ± S.D, P<0.05)

CONCLUSION
From the above studies it can be concluded that Piperine can be used as a formulation additive (Bioenhancer) in oral solid dosage forms of Erythromycin estolate. The inclusion of Piperine as a bioenhancer, will result in better oral absorption of Erythromycin and reduction in its oral dose. The reduction in oral dose will result in minimal side effects, reduced cost of treatment and improved patient compliance.

REFERENCES

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