Formulation and evaluation of solid self micro emulsifying drug delivery system for Clarithromycin

Mrunalini R. Suryawanshi and Manish S. Kondawar

Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India

ABSTRACT

The aim and objective of this study was to develop and characterize self micro emulsifying drug delivery system (SMEDDS) in liquid and pellet forms that result in improved solubility, dissolution and in vitro absorption of the poorly water soluble compound clarithromycin. Solubility of clarithromycin was determined in various vehicles including oil, surfactant and co-surfactant. Pseudo ternary phase diagram were used to evaluate the micro emulsification existence area and the release rate of clarithromycin was investigated using an in vitro dissolution test. SMEDDS formulations were tested for micro emulsifying properties and the resultant micro emulsion were evaluated for clarity, stability, particle size, drug content etc. Formulation development and screening was done based on results obtained from phase diagrams and characteristics of resultant micro emulsion. The solid SMEDDS pellets are characterized by globule size analysis and drug release studies of formulations are compared with plain drug. All batches of liquid SMEDDS was selected for incorporation into MCC in different ratios to assess the possibility of pellet production. Pellets were characterized for their size, shape, friability and in vitro dissolution. The optimized formulation of both liquid and solid SMEDDS showed maximum (80%) release in 60 minutes as compare to conventional tablet of clarithromycin. Thus, the study confirmed that the SMEDDS formulation can be used as a possible alternative to traditional oral formulation of clarithromycin to improve its solubility and bioavailability.

Keywords: self micro emulsifying drug delivery system, clarithromycin, pseudo ternary phase diagram, bioavailability.

INTRODUCTION

The oral route of drug delivery is not possible for approximately 40% of drug compound due to poor bioavailability, solubility, absorption problem, high intra and inter subject variability, high fluctuation in the drug plasma level, variability due to food effect, rapid metabolism, lack of dose proportionality which are playing major role for poor in vivo result leading to failure of conventional drug delivery system. It is a great challenge for pharmaceutical scientist to convert these molecules into orally administered formulation with improves sufficient bioavailability. [1] Recently, much attention has been focused on lipid based formulations to improve the oral bioavailability of poorly water soluble drug. Among the lipid based formulations, one of the formulations is self micro emulsifying drug delivery system (SMEDDS). Self micro emulsifying drug delivery systems are a promising technology to improve the rate and extent of absorption of poorly water soluble drugs.

Self micro emulsifying drug delivery system (SMEDDS) are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants or alternatively one or more hydrophilic solvents and co-solvents/ surfactants that have a unique ability of forming fine oil in water (o/w) micro emulsions (with a droplet size in a range of 10-100nm) upon mild agitation followed by dilution in aqueous media, such as GI fluids. SMEDDS spread readily in the GI tract and the digestive motility of stomach and intestine provide the agitation necessary for self emulsification. [2]
However, traditional preparations of SMEDDS are usually prepared in the liquid state. So the liquid SMEDDS are generally enclosed by soft or hard capsules to facilitate oral administration but it produce disadvantages like high production cost, low drug incompatibility, and stability, drug leakage and precipitation, capsule ageing. So to overcome above problem some solid SMEDDS formulation is prepared from liquid SMEDDS such as SE tablet, capsule, microsphere, pellet, solid dispersion etc. From these we focused our investigation on making pellets of liquid SMEDDS by using MCC as solidifying/adsorbing agent. [3]

Clarithromycin is a macrolide antibiotic. It has elimination half life 3-4 hours and 5-7 hours. The absolute bioavailability of clarithromycin is approximately 50%. Clarithromycin is bcs class II drug. Therefore the main objective of the investigation is to develop and evaluate liquid and solid SMEDDS containing clarithromycin to improve its oral bioavailability by increasing the solubility of drug. From this study we had made an attempt to increase dissolution rate of clarithromycin in order to increase its effectiveness and reduce variability.

MATERIALS AND METHODS

Clarithromycin was obtained as a gift sample from Ind - swift Ltd. samba unit (Jammu and Kashmir). Acconon MC8, 2 EP/NF, Capmul MCM were generous gifts from Abitec Corp., Mumbai. Tween 80, Tween 20, PEG 200, PEG 400 and Microcrystalline cellulose was purchased from research lab, Mumbai. All other chemicals were of reagent grade.

Solubility study:
The solubility study was used to identify the suitable oil and surfactant that possess good solubilising capacity for estimated drug. The solubility of clarithromycin in various vehicles including oils (isopropyl myristate, capmul MCM, Acconon MC8, 2 EP/NF, castor oil, ethyl oleate, olive oil etc.), surfactants (tween 20, tween 80, span 80, span 20 and cremophore RH 40) and co-surfactants (ethanol, PEG 400, PEG 200 and propylene glycol) was determined by shake flask method. An excess amount of clarithromycin was added to each cap vial containing 3 gm of the vehicles. After sealing the mixture was vortexed at a maximum speed for 10 min in order to facilitate proper mixing of clarithromycin with the vehicles. Mixtures were then shaken in shaker maintained at room temperature until equilibrium (48hr). After 24 hour the vial observed for the residue of drug and again the excess amount of drug was added in to the vial showing no residue, and kept for shaking for additional 24 hour. Then mixture were centrifuged at 3000 rpm for 10 min. the supernant were collected into glass vials and analysis was carried with UV-visible spectroscopy to find the concentration of drug. [4]

Pseudo ternary phase diagram:
To obtain an optimum formula of the clarithromycin SMEDDS, which can form a micro emulsion upon dilution with water, pseudo ternary phase diagrams were constructed using the water titration method at ambient temperature. Based on preliminary experiments, Acconon MC8, 2 EP/NF was used as the oil phase, Tween 80 was used as the surfactant and PEG 200 was used as the co-surfactant. The surfactant/co-surfactant ratio used was 1:1, 2:1, 3:1 and 4:1. For each phase diagram, oil and Smix ratio are mixed thoroughly in different weight ratio from 1: 9 to 9:1 (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) to get phase diagram. After clarithromycin was added to the mixture of oil, surfactant and co-surfactant, water was added drop by drop to this mixture with the help of burette. During the titration, the samples were agitated gently with magnetic stirrer in order to reach equilibrium quickly. The phase boundary was determined by observing the changes in the sample appearance from turbid to transparent or from transparent to turbid. All the ratios in this study are reported as weight to weight ratios (w/w). The se values were then used to determine the boundaries of the micro emulsion domain corresponding to the chosen value of oils as well as the S/CoS mixing ratio. The phase diagram was constructed by using chemix software. [5]

Formulation of SMEDDS:
SMEDDS were prepared using tween 80 as surfactant and PEG 200 as cosurfactant in the ratio 4:1 and acconon MC8, 2 EP/NF as oil phase. Formulations of SMEDDS were prepared containing a fixed proportion of clarithromycin (250 mg) dissolved in varying ratio of oil, surfactant and co-surfactant. These components were accurately weighed and mixed using a magnetic stirrer. Depending on solubility, the formulation amount of clarithromycin was dispersed into the mixture of oil, surfactant and co surfactant. Then, the components were mixed by gentle stirring and vortex mixing at 37°C until drug was dissolved completely. Then it was sealed in glass vial and stored at room temperature until used. The composition of formulation was shown in table no.1.
Table no.1: Composition of self micro emulsifying drug delivery system of clarithromycin

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Formulation code</th>
<th>Drug mg</th>
<th>%Composition (W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aconon</td>
</tr>
<tr>
<td>1</td>
<td>M1</td>
<td>250</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>M2</td>
<td>250</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>M3</td>
<td>250</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>M4</td>
<td>250</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>M5</td>
<td>250</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>M6</td>
<td>250</td>
<td>10</td>
</tr>
</tbody>
</table>

On adding MCC as a solid carrier to above SMEDDS formulation and subjected this mixture (MCC + SMEDDS) to extrusion spheronization technique we get pellets or solid SMEDDS formulation.

Characterization of SMEDDS –
Reconstitution properties of SMEDDS

Droplet size and polydispersive index of reconstituted microemulsions: The average droplet size, size distribution and polydispersity index of micro emulsions from liquid SMEDDS were assessed by dynamic light scattering (Brookhaven Instruments, U.K.) at a wavelength of 659.0 nm and a scattering angle of 90° at 25°C. All studies were repeated three times and the average values were used.

Dispersibility Test: [7]
The efficiency of SMEDDS is assessed using standard USP XXIII dissolution apparatus. Each formulation was added to 500 ml of distilled water at 37°C ±0.5°C. The paddle was made to rotate at 50 rpm. The in vitro performance of the formulation was assessed using the following grading system.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Rapidly forms micro emulsion and shows clear transparent appearances.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade B</td>
<td>Rapidly forming, slightly less clear emulsion having a bluish white appearance.</td>
</tr>
<tr>
<td>Grade C</td>
<td>The milky white emulsion like appearance.</td>
</tr>
<tr>
<td>Grade D</td>
<td>Dull, greyish white emulsion is having slightly oily appearances that are slow to emulsify.</td>
</tr>
<tr>
<td>Grade E</td>
<td>Formulation exhibiting either poor or minimal emulsification with larger oil globules.</td>
</tr>
</tbody>
</table>

Speed of Emulsification: [5]
SMEDDS forms rapid o/w micro emulsion in gastrointestinal tract under gentle agitation which provided by digestive motility of stomach and intestine. The rate of formation of micro emulsion is an important index for assessment of formation of micro emulsion. A 1 ml of each pre concentrate of SMEDDS of clarithromycin was diluted to 250 ml with distilled water in a beaker and agitated at 20 rpm the time taken to form emulsion was noted using stopwatch.

Robustness to dilution:
Robustness to dilution was studied by diluting it 100 and 1000 times with various dissolution media viz. 0.1N HCl and buffer pH 6.8. The diluted micro emulsions were stored for 12 h and observed for any signs of phase separation or drug precipitation.

Stability Study:
Freeze thawing
Freeze thawing is employed to evaluate the stability of formulation. The SMEDDS pre concentrate of various formulations were subjected to 3 to 4 freeze thaw cycles, which included freezing at 40°C for 24hr. The various formulations were then subjected to centrifugation at 3000 rpm for 5 min. The formulations were visually observed for phase separation and drug precipitation.

Determination of drug content.[6]
One capsule of each formulation was taken in 100 ml volumetric flask and added 100 ml of pH 6.8 as extracting solvent. This was shaken for 1hr in mechanical shaker and kept aside for 24 hr. After 24 hr, filter the solution through whatman filter paper no 41 and collect the filtrate. The filtrate was then analyzed in Jasco UV spectrophotometer at 275 nm using pH 6.8 as blank. The concentration of drug in solution was calculated from absorbance and standard graph.

In vitro drug release study:
Drug release studies from SMEDDS were performed using USP II, dissolution apparatus II (Lab India–Disso200) with 900 ml of buffer pH 6.8 as a medium at 37 ± 0.5°C. The speed of the paddle was adjusted to 50 rpm. Clarithromycin-loaded SMEDDS were filled into the empty hard gelatin capsule (capsule size 00). Capsules were placed into the egg shell membrane prepared by the treatment of hydrochloric acid. At predetermined time intervals
an aliquot (5 ml) of the sample was collected, filtered and analyzed for the content of clarithromycin by ultraviolet spectroscopy. An equivalent volume (5 ml) of fresh dissolution medium was added to compensate for the loss due to sampling to maintain sink condition.  

**Formulation of pellets**  

**Characterization of pellets** [8][9]  

**Pellet size analysis:** [8]  
Size distribution and shape evaluation of the pellets. Vibrating mechanically for 10 min, a set of Chinese Standard Sieves (1680, 1180, 1000, 850, 710, 420 and 350 µm) were used for size distribution determinations of 10 g of the produced pellets.  

**Friability testing of pellets:**  
Friability testing was conducted using a friability tester. A 10 g pellet sample was placed into the drum together with 10 g glass spheres of 5 mm diameter, and rotated for 10 min at 25 rpm. Pellets were then weighed and friability was calculated according to formula:

\[
\text{% Friability} = \frac{W_0 - W_1}{W_0} \times 100
\]

Whereas, \(W_0=\) Initial weight, \(W_1=\) Final weight

**Micromeritic Properties:**  
The self emulsifying pellets were evaluated for bulk density (BD), tapped density (TD), Compressibility (Carr’s) index and angle of repose. Angle of repose was determined for the measurement of flowability. This was further supported by the value of Hausner’s ratio. The improved flowability of self emulsifying pellets may be due to good sphericity and small size of granules.  

**Scanning electron microscopy (SEM):**  
The surface morphology of the pellets was studied by scanning electron microscopy. An appropriate sample of pellets was mounted on metal (aluminium) stubs. The samples were mounted using double-sided adhesive tape and fractured with a razor blade. The samples were sputter-coated with gold/palladium for 120 seconds at 14 mA under argon atmosphere for secondary electron emissive SEM and observed for morphology at acceleration voltage of 15 KV.  

**Drug content:**  
One capsule of each formulation was taken in 100 ml volumetric flask and added 100 ml of methanol as extracting solvent. This was shaken for 1 hr in mechanical shaker and kept aside for 24 hr. After 24 hr, the solution was filtered through whatman filter paper no 41 and collected the filtrate. The filtrate was then analyzed in Jasco UV spectrophotometer at 275 nm using pH 6.8 as blank. The concentration of drug in solution was calculated from absorbance and standard graph.  

**In vitro drug release test:**  
The drug release test was carried out for 1.5 hr at 100 rpm by paddle method. The dissolution medium was 900 ml buffer (pH 6.8). The temperature of the dissolution medium was controlled at 37±0.5°C. The optimal SE pellets, liquid SEDDS and conventional tablets weighed to be equivalent to 250 mg clarithromycin were used for the dissolution test. Five milliliters of the dissolution medium were sampled at appropriate intervals, and fresh dissolution medium was simultaneously replenished in the apparatus to maintain a constant volume. The withdrawn sample was passed through whatman filter paper no 41 and the filtrate was assayed by UV spectrophotometer at 275 nm to determine the dissolved drug concentration. Each release test was carried out in triplicate.  

**RESULTS AND DISCUSSION**  

**Solubility study**-  
The components used in the system should have high solubilization capacity of the drug in the resultant dispersion. Results of solubility studies are reported in Figure1, 2&3. As seen from figure Acconon MC8, 2EP/NF, Tween 80, PEG 200 showed the highest solubilization capacity for clarithromycin. Thus for our study we selected Acconon MC8, 2EP/NF, tween 80 surfactant and PEG 200 as cosurfactants.
**Pseudo ternary Phase Diagram Study**-

Pseudo ternary Phase Diagram were constructed to identify self micro emulsifying region and to select suitable concentration of oil (Acconon MC8, 2EP/NF), surfactant (tween 80), cosurfactant (PEG 200) for the formulation of SMEDDS. In the present study Acconon MC8, 2EP/NF was tested for phase behavior studies with Tween 80 and PEG 200 as the S/CoS mixture. The micro emulsion area increased as the S/Cos ratios increased. Thus, an S/CoS ratio 4:1 was selected for the formulation study.
Characterization of SMEDDS:

Droplet size and polydisperse index of reconstituted micro emulsions:
The polydispersity values of SMEDDS M1, M2, M3, M4, M5 and M6 are 0.1804, 0.123, 0.131, 0.147, 0.134 and 0.137 respectively, which indicates uniformity of droplet size within the formulation.

Droplet size values of SMEDDS M1, M2, M3, M4, M5 and M6 are 21.33nm, 15.77nm, 15.02nm, 14.94nm, 25.15nm and 17 nm respectively were found having particle size less than 50 nm which fulfill the criteria of micro emulsion and low PDI shows uniformity of particles. The formulation M4 showed the lowest mean particle diameter (14.94nm) whereas M5 showed the highest mean particle diameter (25.15nm). Therefore, SMEDDS M4 was considered as optimized batch.

Dispersibility Test:
The efficiency of self micro emulsification was assessed using dispersibility test. Grade A and grade B formulation remained as micro emulsion when dispersed in GIT, while formulation of grade C is recommended as SEDDS formulation. Formulation M2, M4 and M5 shows clear transparent appearances therefore it rapidly forms micro emulsion thus a system is known a SMEDDS. The formulation batch M1 and F6 shows slightly less clear emulsion having a bluish white appearance, whereas formulation M3 shows the milky white appearance, therefore these systems are termed as self emulsifying drug delivery system.

Speed of Emulsification:
The rate of self emulsification is an important index for assessment of the efficiency of self emulsification. It was observed that M4 showed less dispersion time 25-30 sec. The result showed the order of dispersion time as follows M3(1 min 15 sec) > M5(45-50 sec) > M1(35-40 sec) > M2(30-45 sec) > M6(30-35 sec) > M4(25-30 sec). The decrease
in the self emulsification time of M4 was due to the relative decrease in surfactant concentration leads to decreased viscosity of formulation.

Robustness to dilution:
M3 formulation showed drug precipitation in water, and phosphate buffer where as M5 showed drug precipitation in phosphate buffer and 0.1N HCl. Other Diluted SMEDDS formulation did not show any precipitation on storage in various dilution media. This revealed that M1, M2, M4, M6 were robust to dilution and all M1 to M6 formulation does not show any phase separation.

Stability Study:
Freeze thawing:
Formulation batch M1, M2 showed phase separation upon 3 freeze thaw cycle while rest of all five batches from M3, M4, M5 and M6 were stable for freeze thaw test. This may be because of the lowest concentration of surfactant.

Determination of drug content:
The drug content for all the formulation was found to be in the range of 94.97-99.6% w/w which shows uniformity of formulation. M5 formulation showed high drug content 99.6% w/w while M3 showed low drug content 94.97% w/w and M1, M2, M4, M6 showed 95.83% w/w, 97.07% w/w, 98.07% w/w and 98.62% w/w respectively.

In vitro drug release study:
The dissolution medium phosphate buffer pH 6.8 was used to study the drug release. Result revealed that all batches showed more than 80% of drug released in within 1 hr. It can be observed that there is not much significant difference in drug release between all batches. However batch M4 shows maximum drug release followed by M6 while M1 showed lowest drug release. From this observation we can conclude that percentage of Smix affects the drug release rate as well as extent of release because M4 shows highest concentration (70%) of Smix while M1 have lowest one (40%). The order of drug release decreased as follows: M4 > M6 > M2 > M5 > M5 > M1.

![Fig no. 8: in vitro drug release of SMEDDS](image)

Characterization of pellets [10][11]

Pellet size analysis:
From the results obtained it was found that for batch F1, F2 and F5 majority of pellets were found in the size range of 1680-850 nm i.e. 79.81% and 76.3% respectively; but for batch F3, F4, F6 majority of pellets were found in size range of 1680-1180 i.e. 73.6%. This is because of maximum percent of lipid used for the formulation (40%) and also it can be concluded that increased lipid loads cause a small to moderate, but significant increase of the Ferret diameter and the aspect ratio.

Friability testing of pellets:
The results of the friability test show that the friability was low (less than 1%) for lipid loads up to 30% and 1.82% for the high lipid load of 40%. However, it was found that the friability of the pellets with 40% lipid load were significant higher compared to the lipid load of 20%. Most likely, a low percentage of liquid lipids were strongly bound by MCC. Increasing amounts will most likely be less strongly adsorbed and therefore we taken the interactions within the pellets and increase friability. Result of friability testing of pellet was shown in table no. 2.
EDDS are slightly higher than that of the optimal SE pellets.

The release performance of clarithromycin from SMEDDS formulations (both pellets and liquid SMEDDS) is significantly improved, compared with the conventional tablets. However, the release rate and extent of liquid SMEDDS are slightly higher than that of the optimal SE pellets. The % cumulative drug release of SMEDDS pellets of clarithromycin and conventional tablet of clarithromycin was plotted against time. A comparison of in vitro drug release profile of conventional tablet of clarithromycin and SMEDDS pellets formulation are given in figure no.10.

Micromeritics Properties:

Result of micromeritics properties of pellet were shown in table no. 3.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Batch no.</th>
<th>Friability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>F1</td>
<td>0.84±0.16</td>
</tr>
<tr>
<td>2.</td>
<td>F2</td>
<td>1.16±0.39</td>
</tr>
<tr>
<td>3.</td>
<td>F3</td>
<td>1.28±0.24</td>
</tr>
<tr>
<td>4.</td>
<td>F4</td>
<td>1.02±0.11</td>
</tr>
<tr>
<td>5.</td>
<td>F5</td>
<td>1.82±0.18</td>
</tr>
<tr>
<td>6.</td>
<td>F6</td>
<td>1.04±0.27</td>
</tr>
</tbody>
</table>

All the formulated batches were evaluated for the flow properties and there is no significant change in all batches and F4 show excellent flow but is much higher than rest of five batches. Most likely, a low percentage of liquid lipids are strongly bound by MCC.

Scanning electron microscopy (SEM):

The surface morphology of self emulsifying pellets F4 was studied using SEM.

Details about the surface characteristics of pellets were obtained with increasing the magnification. Figure showed typical surface features of the pellets with an apparently smooth surface.

**Drug content:**

F5 formulation showed high drug content 99.24% w/w while F3 show low drug content 95.21% w/w and F1, F2, F4, F6 showed 96.63% w/w, 96.35% w/w, 98.62% w/w and 98.32 % w/w respectively.

**In vitro drug release test:**

In vitro drug release studies were performed for SMEDDS pellets and clarithromycin tablet. However batch F4 shows maximum drug release followed by F5, F6, F2, F1 and F3, while Marketed formulation showed lowest drug release.

Clarithromycin SMEDDS pellets showed a dramatic improvement in the invitro dissolution profile compared to marketed formulation. Result revealed that clarithromycin SMEDDS pellets showed more than 80% of drug released in 80 min while marketed convensional tablet shows 40%.

The surface morphology of self emulsifying pellets F4 was studied using SEM.

Details about the surface characteristics of pellets were obtained with increasing the magnification. Figure showed typical surface features of the pellets with an apparently smooth surface.

**Drug content:**

F5 formulation showed high drug content 99.24% w/w while F3 show low drug content 95.21% w/w and F1, F2, F4, F6 showed 96.63% w/w, 96.35% w/w, 98.62% w/w and 98.32 % w/w respectively.

**In vitro drug release test:**

In vitro drug release studies were performed for SMEDDS pellets and clarithromycin tablet. However batch F4 shows maximum drug release followed by F5, F6, F2, F1 and F3, while Marketed formulation showed lowest drug release.

Clarithromycin SMEDDS pellets showed a dramatic improvement in the invitro dissolution profile compared to marketed formulation. Result revealed that clarithromycin SMEDDS pellets showed more than 80% of drug released in 80 min while marketed convensional tablet shows 40%.

The release performance of clarithromycin from SMEDDS formulations (both pellets and liquid SMEDDS) is significantly improved, compared with the conventional tablets. However, the release rate and extent of liquid SMEDDS are slightly higher than that of the optimal SE pellets. The % cumulative drug release of SMEDDS pellets of clarithromycin and convensional tablet of clarithromycin was plotted against time. A comparison of in vitro drug release profile of convensional tablet of clarithromycin and SMEDDS pellets formulation are given in figure no.10.
CONCLUSION

From the study it was concluded that, prepared liquid SMEDDS of batch M4 and M6 was robust to dilution, showed dispersibility test of grade A, thermodynamically stable with good self emulsification efficiency and having globule size in nanometric range which is physiologically stable. To overcome problems of liquid SMEDDS, the study was carried out to S-SMEDDS pellets. From the study of S-SMEDDS pellet it was concluded that batch F4 and F6 showed good flow property, drug content and uniform pellet size with low weight loss in friability.

The in vitro drug release of M4 and F4 batch was studied in phosphate buffer of pH 6.8 and showed good drug release as compared to conventional marketed tablet of clarithromycin.

So, SMEDDS improved solubility/dissolution, absorption and bioavailability of poorly water soluble drug clarithromycin.

Acknowledgements:
Author would like to thank Ind swift company, samba unit. (Jammu and Kashmir), for providing gift sample of pure drug clarithromycin and abitec corp. Mumbai for providing gift sample of Acconom MC8, 2EP/NF.

REFERENCES