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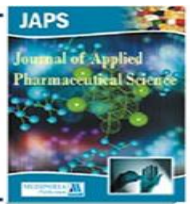

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Dry Suspension Formulation of Taste Masked Antibiotic Drug for Pediatric Use

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ABSTRACT

Dry suspension is commercial dry mixtures that require addition of water at the time of dispensing. The major consequence of the bitter taste is to restrict greatly the further development of oral preparations and clinical applications of these drugs. People wish to take effective drugs that have a nice taste can be administered easily. Accordingly, it is important to mask the unpalatable taste of a drug in order to improve the product quality. The solvent evaporation process is used for microencapsulation which is carried out in a liquid manufacturing vehicle. Eudragit L100 is used as taste masking agent. FT-IR study shows that there is no significant interactions occurring between drug and excipients. The suspension prepared was evaluated for various parameters like sedimentation volume, degree of flocculation, drug content and In-vitro dissolution time. All the parameters were found to be within limits. When the results were compared with marketed preparation suspension was found to be better with respect to marketed preparation.

Keywords: Dry suspension, Taste Masking, Macrolide Antibiotic, Eudragit L 100.

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INTRODUCTION

Clarithromycin (6-0-methyl-erythromycin A) is a 14-membered macrolide antimicrobial agent widely used for treatment of infections such as respiratory infection, skin soft tissue infection, Chlamydia infection and helicobacter pylori infection and so on. It is also clinically active against bacteria responsible for exacerbations of chronic bronchitis and the atypical pathogens that cause respiratory tract infections. Clarithromycin is stable in the gastric acid and well absorbed, but it has a very bitter taste, this make it inconvenient when taken orally. Also used to treat pharyngitis, tonsillitis, acute maxillary sinusitis, acute bacterial exacerbation of chronic bronchitis, pneumonia (KD Tripath**,** 2003). Clarithromycin prevents bacteria from growing by interfering with their protein synthesis. Clarithromycin binds to the subunit 50S of the bacterial ribosome and thus inhibits the translation of peptides. EUDRAGIT® L 100(Degussa, 2009) is anionic copolymers based on methacrylic acid and methyl methacrylate. It is a solid substance in form of a white powder with a faint characteristic odour. It has effective and stable enteric coatings with a fast dissolution in the upper bowel. Granulation of drug substances in powder form for controlled release can be used. Site specific drug delivery in intestine by combination with EUDRAGIT® S grades also shows variable release profiles.

Clarithromycin is a highly bitter taste drug and also it shows the problem of hydrolysis when it is in liquid formulation. This will affect the stability of the suspension and potency of the drug. The purpose of this work is to develop a dry suspension powder from which a permanent stable suspension can be prepared after reconstitution.

MATERIALS AND METHODS

Materials

Clarithromycin, Eudragit L100 was procured from Merck Company Mumbai. Excipients used for preparation of suspension are Sucralose, Aerosil, citric acid, sugar, xanthan gum and colour-quinidine yellow were also procured from Merck Company Mumbai.

Compatibility studies (Beckett *et.al*, 1993)

Fourier transforms infrared spectroscopy (FTIR) analysis

Drug and polymer were mixed in ratio of 1:1 and mixtures were placed in sealed vials for 3 months at room temperature. FTIR measurement of drug and individual polymer and drug polymer mixture were obtained on Simadzu FTIR. Samples were prepared with Kbr and placing in sample holder. The spectra were scanned over the wave number range of 4000-400 cm−1 at the ambient temperature.

Preparation of Clarithromycin microspheres (Lachman *et.al*, 1990)

Taken Acetone required to disperse the accurately weighed quantity of polymer Eudragit L100 in it. Stirred for few min and then added weighted quantity of Clarithromycin, stirred for 10-15 min with 4000 rpm to ensure uniform dispersion of drug particles. Transferred the microcapsule in tray and allowed drying on room temperature for 24 hours to ensure complete evaporation of acetone. Microcapsules were crushed and then passed through sieve # 80 to get the uniform, free flowing and discreet particles. The microcapsules were separately prepared using polymer Eudragit L100 in Drug: Polymer ratio 1:1, 1:2, 1:3, 1:4 and 1:5 following the same procedure. Drug proportion was maintained constant so as to determine maximum polymer required to encapsulate drug, and to obtain desired drug release.

Evaluation of microcapsules

Particle size and morphology evaluation (Martin Alfred, 1994)

Optical microscope was used to evaluate both the morphology and surface characteristics of the microcapsules.

Micromeritic properties (Fonner et.al.1993)

The granule size separation was carried out by vibrating sieve shaker at medium vibration level for 20 min using five standard sieves of #8 (2360 µm) to #200 (75 µm). Undersize fractions (fines collected in collector was excluded) were then collected, stored in a desiccators at 25 ± 2˚C and used for the dissolution studies. Powder properties of all preparations were measured using tap density tester.

Drug loading rate and entrapment rate

Validation of the optimized HPLC method was carried out with respect to the following parameters.

Chromatographic conditions

The HPLC separation was performed using a methanol as a mobile phase, consisting of potassium dihydrogen phosphate (KDP, 0.067 mol/l) acetonitrile (35:65), was delivered at a flow-rate of 1.0 ml/min; Detection was set at 220 nm and the column temperature was maintained at 50°C. Stop time 15.0 mins.

Standard Calibration Curve of Clarithromycin:

Preparation of working solution for standard calibration curve A 10 ml (1000µg/ml) of stock solution was diluted to 100 ml with methanol to get 100 µg/ml solutions. From above mentioned working standard solution, aliquots was taken and then diluted up to 10 ml with methanol to get 10-100ug/ml.

Sample analysis

Weighted 80 mg of microcapsules accurately, dissolved 20ml of mobile phase using the ultrasound, transfered the content to 50 ml volumetric flasks and make up the volume with mobile phase. Filtered sample with 0.2u filter paper and used the filtrate for further analysis. Taken 20 µl of filter liquor accurately, injected and calculated the content (Liandong Hu *et.al*, 2011).

Drug Loading =

$$\frac{\text{Weight of the drug loaded in a microcapsule X 100}}{\text{The total weight of microcapsules}}$$

Microencapsulation Efficiency =

$$\frac{\text{Estimated percent drug content X 100}}{\text{Theoretical percent drug content}}$$

The pure drug and taste masked microcapsules was compared for its taste and from that it is decided whether the taste of the drug is to be masked or not.

Percent Production Yield

The percentage production yield of the produced microcapsule can be calculated for each batch by dividing the weight of microcapsule (M) by the total expected weight of drug and polymer (Mt).

%Production Yield= M/Mt

In Vitro Dissolution Testing

The USP apparatus 1 (rotating basket) set to sink conditions has been used to study in vitro drug release. Microcapsules equivalent to 500 mg of Clarithromycin were placed in basket of dissolution test apparatus in 0.1 N HCL sink condition for 2 hours. Dissolution medium was maintained at 37 $\pm$ 0.5 .After 2 hours 900 ml of phosphate buffer 6.8 was replaced and used to carry out dissolution with the basket at 100 rpm. 5 ml of aliquots were withdrawn by single mark pipette after every 30 min interval and volume withdrawn was replaced with fresh equal quantities of

fluid. Taken filter liquor and make sufficient dilution and determined for the drug release by HPLC and then calculated accumulative drug release (Sandile et. al.2009).

Formulation of Dry Suspension of Clarithromycin

Microcapsules of optimized ratio i.e. 1:3 were selected to formulate dry suspension. Dry suspension was formulated as per following formula (Table. 1) all ingredients were passed through 40# sieve. The smallest amount of drug is mixed with same amount of other excipients.. All the ingredients were mixed thoroughly flavour was added at the end. The dry suspension which is formed is reconstituted up to 30 ml with distilled water before use (Hirali K Shah *etal*., 2010).

Table. 1: List of excipients used for formulation of suspension.

Ingredients	Weight (gm)
Complex	3.01
Sucralose	0.045
Aerosil	0.090
Citric acid	0.071
Xanthan gum	0.096
Color (quinidine yellow)	0.0040
Sugar upto	Qs to17gm

Each formulation is equivalent to 0.75 gm of Clarithromycin. 5ml of suspension contain 125 mg of Clarithromycin.

Evaluation of dry suspension

Assay for Drug content

Sample solution:

Taken 3.45g of sample (Clarithromycin suspension) which is equivalent to 3.0 ml in 50 ml beaker, added 20 ml of mobile phase and stirred for 30 minutes to dissolve, transferred the content to 50 ml volumetric flasks and made up the volume with mobile phase, filtered with 0.2u filter paper and used the filtrate for further analysis.

Chromatographic condition:

- Mobile phase - Methanol: 0.2M KH2PO4 (35:65)
- λ max - 220nm
- Temperature - 50 ^{0}C
- Flow rate - 1.0 ml/min
- Stop time - 15.0min

Sedimentation Volume

The sedimentation volumes were determined by keeping 50 ml of each suspension in stopper measuring cylinder and stored undisturbed at room temperature. The separation of clear liquid was noted at intervals of 1 day and up to 14 days.

The sedimentation volume *F* was calculated using the formula $F = Vu/Vo$, where *V*u is the volume of sediment and *V*o is the original height of the sample .It is expressed as a percentage (Sateesha *et.al*, 2010).

Degree of flocculation (β) (Gohle *et.al*, 2007)

$$\beta = F/F\infty = \frac{V_u/Vo}{V\infty/V_o} = Vu/V_\infty$$

$$= \frac{\text{Ultimate sediment volume fo flocculated suspension}}{\text{Ultimate sediment volume fo deflocculated suspension}}$$

Stability studies (Kulkarni *et.al*, 2004)

Drug content determination

The chemical stability of Clarithromycin is important because the physicochemical characteristics of Clarithromycin depended on excipients employed in preparation. Hence the preparations was subjected for stability studies. The stability of Clarithromycin was assessed by evaluating the percentage of the initial concentration remaining after specific period of time under different conditions. A difference in concentration by ± 10% was considered a notable change in drug stability.

pH measurements

Change in pH of the suspension followed by reconstitution was measured for all the formulations using a digital pH meter on day 1 and day 14 at 25˚C.

Comparison with marketed preparation

In vitro release of prepared suspension and marketed preparation is compared. The condition for the dissolution study is same as that of microcapsule.

RESULTS AND DISCUSSION

FTIR Studies

When IR of Clarithromycin (Fig 1) was correlated with physical mixture of drug and excipients (Fig 2) the region of 3410 cm^{-1} was found due to the N-H (aromatic) stretching. However other peaks related to C-H, C-O and carbonyl stretching remain unchanged (Table No. 2). This indicates that overall symmetry of the molecule might not be significantly changed, therefore the FT-IR study revealed that there is no interactions taking place between Clarithromycin and Eudragit L100.

Table. 2: FT-IR study of Clarithromycin and Physical mixture of Clarithromycin -Eudragit L100.

Material	Functional group	FT-IR signalling (cm^{-1})
Clarithromycin	Tertiary –N stretching.	3468
	Carbonyl stretching	1734
	Aliphatic –CH stretching.	1172
	Hydroxyl (OH) stretching.	2941
Clarithromycin and Eudragit L 100 complex	Tertiary–N stretching (aromatic).	3410
	Carbonyl stretching	1734
	Aliphatic –CH stretching.	1172
	Hydroxyl (OH) stretching	2941

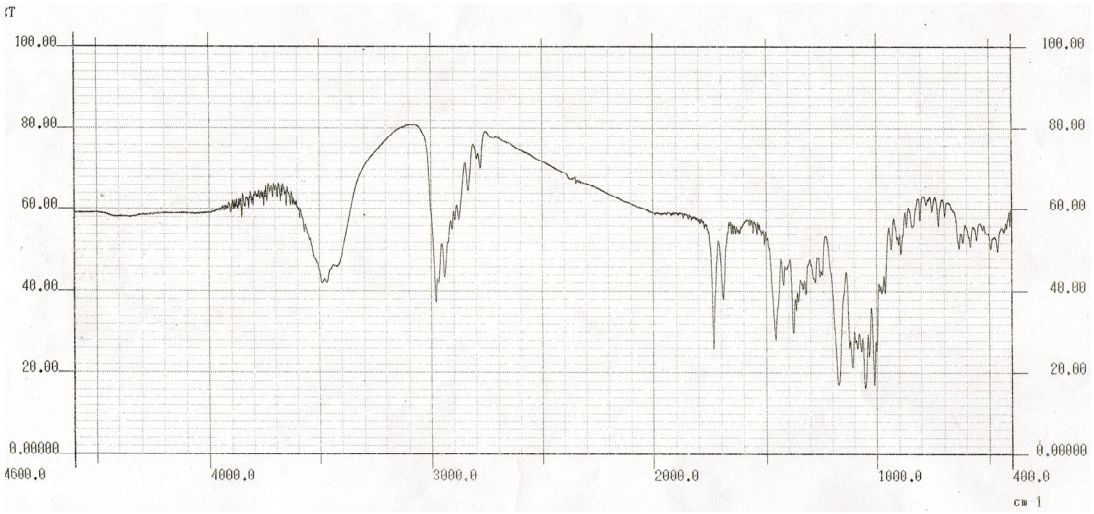

Fig. 1: IR Spectrum of Clarithromycin.

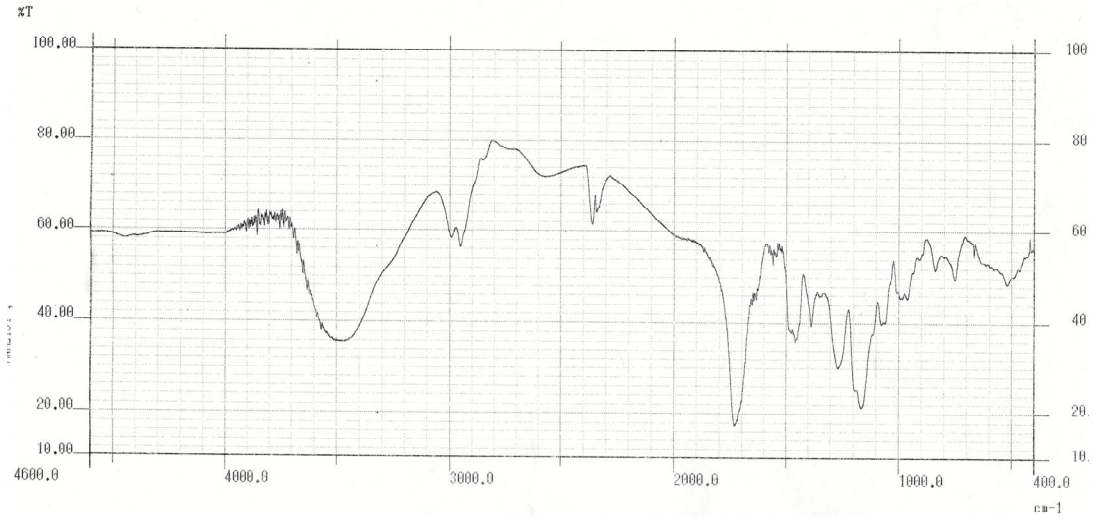

Fig. 2: IR Spectrum of Microcapsules

Standard calibration curve of Clarithromycin

Clarithromycin was found to be soluble in organic solvents such as methanol. HPLC method of estimation was carried out in methanol ranging from 10-100 mcg/ml solutions at 220 nm against the blank. The standard graph obtained was linear, with regression coefficient 0.999as indicated in (Fig 3).

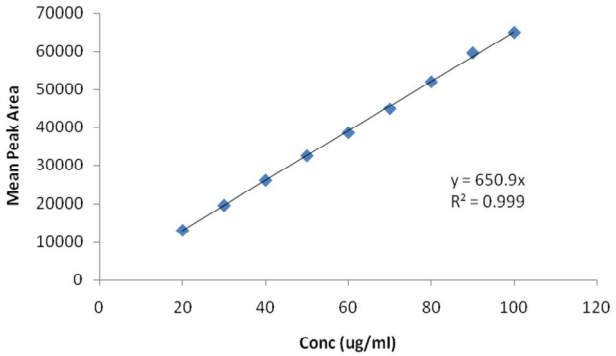

Fig. 3: Standard calibration curve of Clarithromycin.

Evaluation of Microcapsules

It can be observed from the (Table 3)that all the batches of microcapsules have bulk density are less than 1.2gm/cm^3 and angle of repose less than 40º C indicates good flow properties.

Table. 3: Physical property of microcapsules.

Ratio Clarithromycin : Eudragit L 100	Bulk density (gm/cm^3)	Angle of Repose
1:1	0.33 ± 0.010	30.30 ± 0.11
1:2	0.32 ± 0.012	29.52 ± 0.22
1:3	0.34 ± 0.016	27.54 ± 0.12
1:4	0.41 ± 0.013	31.53 ± 0.21
1:5	0.45 ± 0.018	26.80 ± 0.18

Percent Drug Loading, Encapsulation Efficiency and Production Yield of Different Batches of Microcapsules

Drug content of prepared microcapsules was determined by procedure described earlier. The drug content of all 5 formulations and encapsulation efficiency of microcapsules and percentage production yield is tabulated in (Table 4).

Table. 4: Percent Drug Content and Encapsulation Efficiency and Production Yield of Different Batches of Microcapsules

Ratio of Clarithromycin: Eudragit L100	%Drug Loading	Encapsulation efficiency	% Production Yield
1:1	42.80	85.6	95.34
1:2	29.56	88.68	96.56
1:3	23.15	92.60	97.56
1:4	18.88	94.4	99.98
1:5	9.85	96.20	97.21

In Vitro Drug Release Profile

The drug release profiles of different batches of microcapsules were studied in phosphate buffer 6.8. The results obtained for different of microcapsules are tabulated in following. (Table 5). From the (Fig 4) it was found that microcapsules of 1:3 ratio show better release with excellent taste masking with minimum concentration of Eudragit L100. Hence 1:3 batch was selected for formulation of dry suspension.

Tables. 5: In vitro release profile of microcapsules.

Time in min	% drug release of microcapsules of different Ratios				
	1:01	**1:02**	**1:03**	**1:04**	**1:05**
0	0	0	0	0	0
30	6.01	6.34	5.9	5.6	5
60	17.25	15.24	14.99	18.67	15.67
90	34.29	26.78	22.87	25.23	21.98
120	45.29	39.24	32.67	29.45	24.98
150	54.34	45.23	47.86	39.87	31.37
180	62.78	52.98	55.45	45.29	39.45
210	67.69	65.26	64.67	50.35	45.68
240	79.35	78.93	70.45	53.68	49.76
270	83.68	82.45	75.34	58.49	52.69
300	90.34	86.79	79.26	60.45	56.78
330	87.45	85.23	83.67	60.48	53.64
360	87.56	86.41	85.56	61.45	55.16
390	87.91	87.34	85.91	62.43	55.67
420	88.34	87.91	86.54	62.9	56.24
450	89.01	88.3	87.24	63.5	56.82

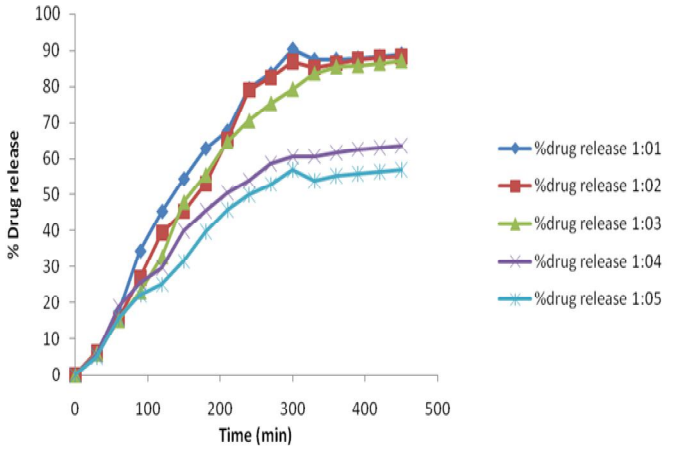

Fig. 4: In vitro drug release profile of five batches of microcapsules

Optimization of Microcapsules

From the in vitro release profiles of all the batches of microcapsules prepared with Eudragit L100 in ratio of 1:3 shows required rate of drug release .Also this batch showed encapsulation efficiency (92.60%) (Table 4), it stood well in all physical evaluation parameters. Hence this batch was selected for further studies.

Surface Characterization

The microcapsules were scanned using scanning electron microscope. The scanning electron micrograph (SEM) of microcapsules showed that microcapsules were spherical in shape with the presence of rough porous polymeric film. (Fig 5 a & b) .

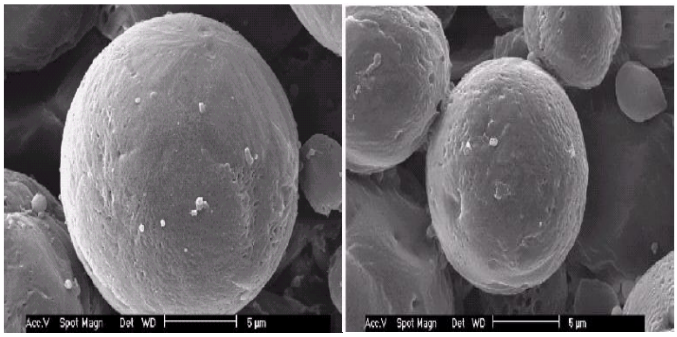

Fig. 5: Scanning Electron Micrograph of Optimized Microcapsules at 60 X Magnification

Evaluation of oral reconstitutable suspension

Assay value of the suspension was found to be 97.73%.

Sedimentation volume

From the figure it is observed that the sedimentation volume is between 1-0 at the end of 14 days, it shows good stability of suspension .The shape of curve shows good stability of suspension (Fig 6).

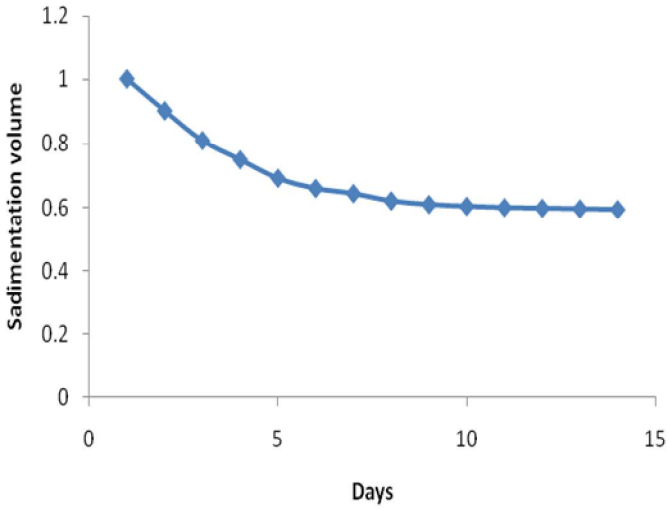

Fig. 6: Sedimentation Volume.

Degree of flocculation

β=28/25

β=1.12 Shows the greater stability**.**

If the **β** value is near to 1, then the suspension does not represent a flocculated suspension. It indicates that the system under study is deflocculated system. But **β** can assume any value greater than 1.In general, the higher the value of B, the greater is the physical stability.

Stability studies

There is no significant change in pH and Drug content of suspension. The prepared formulation show good stability for 14 days (Table 6)

Table. 6: Physical properties of reconstituted Suspension of optimise batch.

Optimise batch formulation (1:3)	pH of the formulation at 25^0C	Drug content%
Day 1	4.42	95.04
Day 14	4.40	93.09

Comparison with marketed preparation (Clarie)

The release profile of prepared Clarithromycin suspension was better as compared to marketed suspension as shown in (Fig 7).

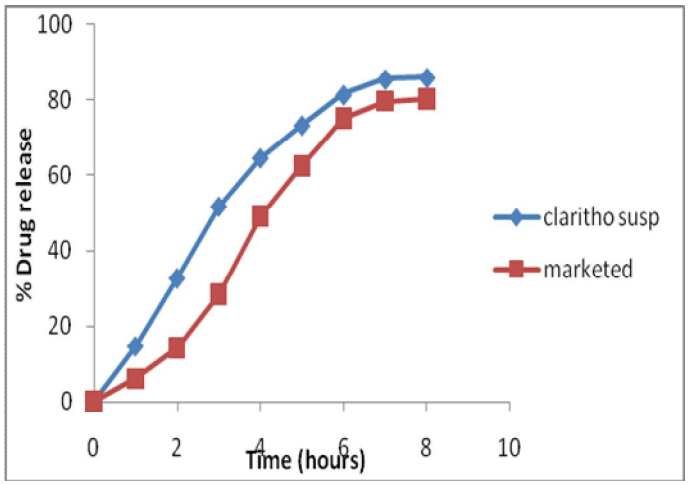

Fig. 7: In vitro release comparison with marketed preparation.

Table. 7: In vitro drug release of formulation compared with marketed preparation (Clarie).

Time(hours)	Clarithromycin suspension	Marketed Suspension (Clarie)
0	0	0
1	14.66	6.1
2	32.67	14.1
3	51.68	28.23
4	64.67	48.9
5	73.4	62.34
6	81.45	75.13
7	85.56	79.68
8	85.97	80.23

CONCLUSION

In present study microcapsules of Clarithromycin was prepared by using different ratios of Clarithromycin to Eudragit L100. Eudragit L 100 shows good taste masking property for Clarithromycin at 1:3 drug and polymer ratio. Also show better drug release profile for Clarithromycin. Prepared suspension of Clarithromycin shows better taste as compared to marketed preparation.

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