



Research Article

STUDIES ON READYMIX SUSPENSION OF AMPICILLIN TRIHYDRATE: DEVELOPMENT, CHARECTERIZATION AND IN-VITRO EVALUATION

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ABSTRACT

Ampicillin trihydrate is used as an antibacterial agent, with an oral dose of 250-500 mg three to four times a day. Reconstitutable Ampicillin trihydrate dry syrup is currently available in the market, where reconstitution of the product has to be done by the consumer, which may lead to handling errors. In addition, the shelf life of reconstitutable dry syrup is only for about a week after reconstitution. Ampicillin trihydrate was attempted to formulate into ready mix oral suspension with improved stability and shelf life. In the first approach of preparation, water was used as suspending medium and pH of the formulations was chosen is in the range of 5 to 6.5. In the second approach, oils like fractionated coconut oil and refined sunflower oil were used as suspending media. The content uniformity of the prepared formulations was analyzed and found to be within the limits. Physical characteristics like sedimentation volume, ease of redispersability and viscosity were evaluated. Particle size determination revealed that majority of the particles was in the size range of 15- 75 μm . *In vitro* dissolution studies were carried out and all the formulations showed 100% dissolution at 50th minute. Stability studies were carried out at 25°C/60% RH and 30°C/60% RH for 90 days. The drug content was analyzed on 7th, 14th90th day on an interval of 7 days. Sedimentation volume, viscosity, ease of redispersability, particle size distribution and *in vitro* dissolution were analysed on 1st and 90th day. Formulation FI and FIV showed

Considerable amount of drug degradation. All other formulations did not show appreciable changes when evaluated. Ampicillin trihydrate degradation during the accelerated stability studies was carried out for 30th day sample using TLC method. It was found that the R_f value of Ampicillin trihydrate in both standard solution as well as formulation was found to be same. This confirmed that there was no degradation of Ampicillin. Hence it was concluded that Ampicillin trihydrate could be formulated into ready mix oral suspension with improved stability and optimum dissolution characteristics.

Keywords: Ampicillin trihydrate, Reconstitutable, Oral suspension, Stability studies

INTRODUCTION

A substantial number of drugs formulated in the form of ready mix oral suspension have been introduced into market.

Oral route of drug administration has been used for decades, which is preferred to be most convenient and easy. Hence it is most widely used among all routes of drug administration. Suspensions though have to undergo dissolution are still advantageous over solid dosage forms as disintegration step is absent and the drug is ready for solubility in the gastro intestinal medium. Because of this suspensions are widely used for oral route of administration¹

Ampicillin trihydrate is a semi- synthetic penicillin derivative, and having an antibacterial spectrum broader than that of penicillin – G has been attained. It is active against gram-positive organisms that are susceptible to other penicillins and it is more active against some gram negative bacteria and enterococcal infections

When Ampicillin is given orally, it is absorbed from intestinal tract to produce peak blood level concentration in about two hours. Ampicillin trihydrate acts on microorganism by interfering with development of bacterial cell wall. Specifically, they inhibit biosynthesis of dipeptidoglycon that is needed to provide strength and rigidity to bacterial cell wall². Seham A.Elkheshen, Sabry, S.Budawi and Alin-A.et al., have done work on optimization of a reconstitutable suspension of Rifampicin. They have derived a very easy and a simple method for estimating the ease of redispersibility³. J.M.Hempens tall, Irwin WJ, Wanpo, Ali, and Andrew A.H. have done study on "Antibiotic granules for reconstitution as syrup" where the drugs chosen were Phenoxy methyl penicillin and Ampicillin. They have reported that the stability of the suspension depends mainly on the suspending medium used⁴.M.R.Vora, Patel M.M, Gohel M.C and Chauhan G.M. have done study on "Formulation of Timidazole suspensions". They have reported that the excipients used in the preparation of a suspension play a very vital role in its normal stability, as well on the photostability of the suspension⁵. Ampicillin trihydrate has been attempted to formulate in ready mix oral suspension. The existing Ampicillin dry syrup has to be reconstituted before use. The direction given for dry syrup is to reconstitute by adding purified water up to the mark given on the label, which is to be done by the user only. Because of faulty label it may affect the dosage regimen. To avoid this problem an attempt is made in the present investigation to prepare ready mix oral suspension of Ampicillin with improved shelf life.

EXPERIMENTAL

MATERIALS AND METHODS

Ampicillin trihydrate is obtained as a gift sample from K.A.P.L – Peenya, Bangalore, Carboxy methylcellulose sodium (Loba chemie Pvt. Ltd.)Aerosil (SmithKline Beecham, Mysore) Tartrazine Colour (Hi-media, India.)Sodium benzoate (Ranbaxy lab-ltd.)Sodium acetate (Ranbaxy fine chemicals Ltd).Ninhydrin reagent (Loba chemie Pvt. Ltd.)Pineapple Flavour (Genuine chemicals Co, Mumbai). Citric acid (Ranbaxy fine chemicals Ltd). Sodium hydroxide (Ranbaxy fine chemicals Ltd) Fractionated Coconut Oil (Trans World Oils Pvt. Ltd, Kerala.)Refind Sunflower Oil (Sunpure Pvt. Ltd., Mysore.)

Method of preparation of suspension

Trial and error method was followed to reach the optimum formulation using different quantities of excipients. The various formulations that were prepared are listed in (Table-1).

All the ingredients were added in geometric proportions. Preparations then transferred to homogeniser and homogenized for 15minutes. Finally volume and pH were adjusted wherever required⁶.

Assay for drug content

Exactly 1ml of suspension was transferred to 100ml volumetric flask and volume was made to 100ml with 5N-Sodium hydroxide, from this 1ml was withdrawn , transferred to a 10ml volumetric flask and volume was made to 10ml with 5N-Sodium hydroxide. The amount of drug present in the above solution was analyzed by measuring the absorbance at 272nm⁷.

Sedimentation volume⁸

Sedimentation volume of the formulations was determined using following formula.

$$V_s = H_u / H_0$$

V_s= Sedimentation volume, H_u= Ultimate settled height of suspension, H₀=Original height of the suspension before settling

Ease of redispersibility³

The suspension was allowed to settle in a measuring cylinder. The mouth of the cylinder was closed and was inverted through 180° and

the number of inversions necessary to restore a homogeneous suspension was determined. If the homogeneity of the suspension was attained in one inversion, then the suspension was considered 100% easily redispersible. Every additional inversion decreases the percentage of ease of redispersibility by 5%.

Viscosity determination

The viscosity of all formulations was determined by using Brookfield digital viscometer. The measurements were carried out using spindle number-3 (disc type) rotating at 10, 20, and 100 rpm. The temperature was maintained at 30°C.

Table 1: Composition of Ampicillin trihydrate ready mix oral suspensions

Formulation	I	II	III	IV	V	VI
Ampicillin trihydrate	6.25%	6.25%	6.25%	6.25%	6.25%	6.25%
Carboxy methyl cellulose sodium	1.83%	1.83%	1.83%	1.83%	1.83%	1.83%
Aerosil	0.75%	0.75%	0.75%	0.75%	0.75%	0.75%
Tartrazine (color)*	q.s	q.s	q.s	q.s	q.s	q.s
Sodium Benzoate	0.45%	0.45%	0.45%	0.45%	0.45%	0.45%
Sugar	90%	90%	90%	90%	90%	90%
Pharmaceutical grade						
Pineapple flavor*	q.s	q.s	q.s	q.s	q.s	q.s
Suspending medium*	Water q.s	Water q.s	Water q.s	Water q.s	Fractionated coconut oil q.s	Fractionated coconut oil q.s
pH	5.0	6.0	5.5	6.5	-	-
Citric acid	q.s	q.s	q.s	q.s	q.s	q.s

* Quantity sufficient

Particle size distribution ^{9,10}:

Using optical microscope particle size distribution studies were carried out.

1. Eye piece micrometer was calibrated using stage micrometer,
2. Sample was uniformly suspended in paraffin oil.
3. A slide of above suspension was prepared, placed under microscope and measured the size of the particles.

In vitro test of dissolution

Prepared suspension formulations were subjected for dissolution using a USP (XXII) rotating paddle dissolution apparatus (apparatus II). The dosage forms were placed in 900ml of distilled water as a medium at 37^o(± 1^o C). The media was agitated by paddle rotating at 100 ± 2 rpm. Aliquots of 10ml of dissolution medium were drawn at intervals of 10th, 20th, 30th, 40th, 50th and 60th minutes. An equivalent volume of fresh dissolution medium was added in the dissolution vessel after each sample withdrawing. The percentage of drug dissolved was determined by measuring the absorbance at 320nm^{11, 12}.

Accelerated stability studies

The prepared formulations were stored at 25°C/60%RH and 30°C/60%RH. Samples from the stored preparation were taken and analyzed after every 7th day for the period of 90 days for drug content uniformity calculations.

At the end of the 90th day samples were analyzed for viscosity, redispersibility, sedimentation volume, drug content and *in vitro* dissolution profile

TLC Studies

TLC plates were prepared by using silica gel G as a stationary phase and 3% w/v solution of sodium acetate in water as mobile phase, developing the plates in a saturated chamber. Spraying 5% w/v solution of ninhydrin identified the spots. Ampicillin trihydrate pure drug was used as standard. The R_f values were calculated for standard and sample.

RESULTS AND DISCUSSION

The prepared ready mix formulations of ampicillin trihydrate were found to possess an excellent redispersibility property with optimum particle size distribution. Sedimentation studies showed that the sedimentation volume of all formulations is below 1, which indicates that the formulations were optimum and acceptable. The viscosity of all the formulations was such that it would be easily pourable from the container and also showed a shear thinning effect. The percentage drug content of the prepared suspension was within the standard limits of the pharmacopoeia. Results of the Comparative evaluation of Ampicillin trihydrate suspension formulations are shown in Table-2.

Dissolution of the prepared formulations proved that ampicillin trihydrate release from all the formulations was almost similar with 100% dissolution within 50 minutes (Fig1&2)

Table 2: Comparative evaluation of Ampicillin trihydrate suspension formulations

Parameters Evaluated	F-I	F-II	F-III	F-IV	F-V	F-VI
Appearance	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
Viscosity (mps100rpm)	373	315	320	310	2850	2901
Sedimentation Volume (After 24hrs)	0.86	0.84	0.84	0.81	0.77	0.76
Redispersibility (%)	95	95	95	95	90	90
Particle size range (µm)	15-250	15-250	15-250	15-250	15-250	15-250
Drug content (%)	104±0.12	102.1±0.08	104±0.09	104.1±0.04	104.2±0.04	102.2±0.09
In-vitro%drug release(After 50 mins)	100.55	100.49	100.76	100.96	100.67	100.25
R _f Value	0.95	0.95	0.95	0.95	0.95	0.95

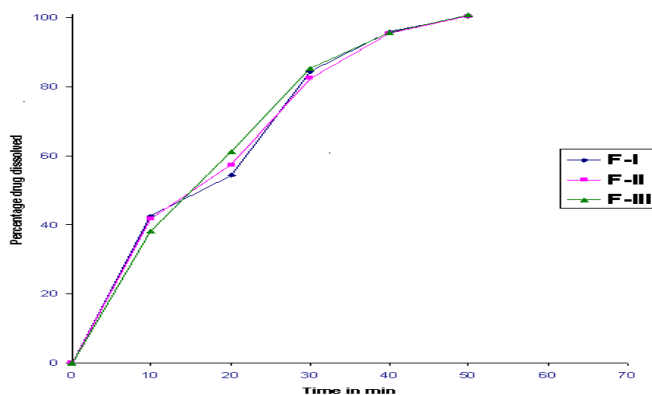


Fig. 1: Percentage of drug dissolved Vs time for formulation F-I, F-II&F-III

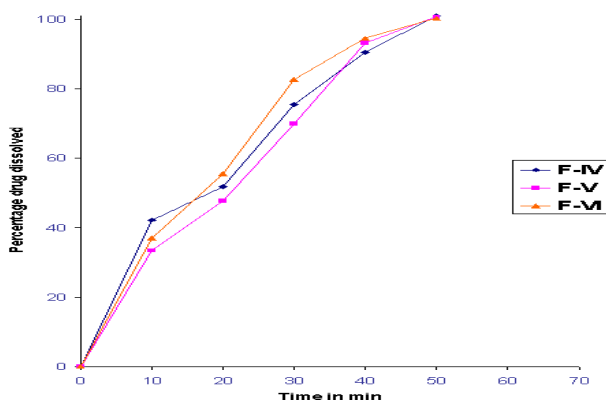


Fig. 2: Percentage of drug dissolved Vs time for formulation F-IV, F-V&F-VI

Accelerated Stability Studies at 40°C/ 75% RH

Results are cited in the table. Data in Table 3(a) and 3(b) shows that the drug is not stable at 40°C and 75% RH. As it is clear from the table that within 15 days the drug is degraded below the pharmacopoeial limits. According to the ICH- guidelines¹³ if there is any significant change like 5% potency loss or degradant exceeds specification limits or other parameters which fail to meet the specifications or if significant change occurs at 40°C / 75%RH, then stability testing is to be carried out at 30°C /60% RH. So testing was carried out at 30°C /60% RH and an additional study was carried out at 25°C /60% RH. Formulations F-II, F-III, F-V and F-VI were stable and no significant change was observed with respect to

percentage drug content, viscosity, ease of redispersibility, particle size distribution and drug dissolution after and during accelerated stability studies. Formulations F-I and F-IV showed a significant decrease in percentage drug content after 70th (95.7%±0.12 & 96.2%±0.04) and 77th (94.6%±0.12 & 94.12±0.16) day respectively. But it was observed that the physical and dissolution properties of these formulations remained unaltered. TLC results showed that the R_f value of Ampicillin trihydrate in both standard solution as well as formulation was found to be same. This confirmed that there is no degradation of Ampicillin. Based on above observations, it can be concluded that Ampicillin trihydrate can be formulated as ready mix oral suspension with improved shelf life.

Table 3(a) Results of % drug content of formulations F-I, F-II, and F-III.

Time in days	Label claim	% Drug content (Mean ± S.D*)		
		F-I	F-II	F-III
1 st day	25mg/ml	104±0.08	104.2±0.08	104.3±0.04
7 th day		98.1±0.16	99.1±0.08	96.8±0.08
14 th day		64.1±0.14	68.1±0.14	72.6±0.12

* Standard deviation N=3

Table 3(b) Results of % drug content of formulations F-IV, F-V and F-VI.

Time in days	Label claim	% Drug content (Mean ± S.D*)		
		F-IV	F-V	F-VI
1 st day	25mg/ml	102.3±0.09	102.4±0.04	104±0.08
7 th day		97.6±0.12	96.5±0.16	97.33±0.12
14 th day		59.9±0.12	68.6±0.87	76.1±0.04

* Standard deviation N=3

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