International Journal of Applied Pharmaceutics
Vol 2, Issue 3, 2010

Research Article

FORMULATION AND *IN VITRO* EVALUATION OF READYUSE SUSPENSION OF AMPIILLIN TRIHYDRATE

AEJAZ AHMED*, ASGAR ALI

Luqman College of pharmacy, old jewargi -road, Gulbarga, Karnataka, India

Received: 02April 2010, Revised and Accepted: 08 May 2010

ABSTRACT

Large number of drugs comes in dry syrup form available in the market, where reconstitution has to be done by consumer, which may lead to handling error. Ampicillin trihydrate used as an antibacterial agent, has poor stability in presence of water having shorter shelf life of about a week upon reconstitution. Hence attempted to formulate in to rediuse oral suspension with improved stability and shelf life. In the first approach ,water was used as suspending medium and pH of formulation was chosen in the range of 5 to 6.5.In second approach fractionated coconut oil and refined Almond oil were used as suspending media. the content uniformity of formulation was found to be within the limits physical characteristics like viscosity ,redispersibility sedimentation volume were evaluated. Particle size determination revealed that majority of particles was in the size range of 15-75mm.all formulations showed good dissolution profile with 100% dissolution at 50th minute. Viscosity, redispersibility and sedimentation volume and particle size distribution and *invitro* dissolution were analyzed on .1st and 90th day .Formulation F-I &F-IV showed considerable amount of drug degradation. All other formulations did not show appreciable changes when evaluated. Accelerated stability studies of rediuse Ampicillin trihydrate suspension carried out for 30th day sample using TLC method. The RF value of standard solution and 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 rediuse oral suspension with improved stability.

Keywords: Ampicillin trihydrate; reconstitutable;oral suspension; stability studies

INTRODUCTION

The oral route of drug administration is the most common for systematically acting drugs. For decades liquid formulations are preferred in use of pediatric and geriatric patients because of the flexibility in administration of usually large doses, safety and convenience of administration.

Suspension though have to undergo dissolution are still advantageous over solid dosage forms as disintegration step in absent and drug is ready for solubility in the gastrointestinal medium. Because of this suspension are widely used for oral route of administration[1].

Suspensions are generally coarse dispersions in which insoluble drug particles are dispersed in a liquid medium[2].Some authors also refer as preparation containing finely divided drug particles(referred to as suspensiod)distributed somewhat uniformity throughout a vehicle in which the drug exhibit a minimum degree of solubility .

Commonly available suspensions ,which are given orally are of antibiotics ,antacids and analgesics[3].A large number of drugs formulated in the form of readiuse oral suspensions have been introduce in to the market which overcome the improper dilution problem associated with erroneous labeling.

Ampicillin trihydrate is a semi synthetic penicillin derivative, having an antibacterial spectrum broader than that of penicillin active against gram positive organisms that are susptable to other penicillin and it is more active against some gram negative bacteria and enterococcal infections given with an oral dose of 250-500 mg three times a day.Ampicillin trihydrates acts on microorganisms by interfering with development of bacterial cell wall.Specially,they inhibit biosynthesis of dippeptidoglycon that needed to provide strength and rigidity to bacterial cell wall[4] .

The existing Ampicillin dry syrup available in market has to be reconstituted before use has poor stability in presence of water, the available formulation guarantee a short shelf life upon final preparation after reconstitution with water is only about a week.Even for reconstitution of dry syrup with 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 to prepare readiuse oral suspension of Ampicillin with improved shelf life.

MATERIALS AND METHODS

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

Method of preparation of suspension

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

All the ingredients were added in geometric proportions. Preparations then transferred to homogenizer and homogenized for 15minutes. Finally volume and pH were adjusted wherever required [5].

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 272nm[6].

Sedimentation volume[7]

Sedimentation volume of the formulations was determined using following formula.

$$Vs = \frac{Hu}{H_0}$$

Vs = Sedimentation volume

Hu = Ultimate settled height of suspension

H_0 = Original height of the suspension before settling

Table 1: composition of Ampicillin trihydrate readiuse oral suspensions

Formulation						
Ampicillin trihydrate	6.25%	6.25%	6.25%	6.25%	6.25%	6.25%
Carboxy methyl	1.83%	1.83%	1.83%	1.83%	1.83%	1.83%
Cellulose sodium	0.75%	0.75%	0.75%	0.75%	0.75%	0.75%
Aerosil	q.s	q.s	q.s	q.s	q.s	q.s
Tartrazine (color)*	0.45%	0.45%	0.45%	0.45%	0.45%	0.45%
Sodium Benzoate	90%	90%	90%	90%	90%	90%
Sugar Pharmaceutical grade	q.s	q.s	q.s	q.s	q.s	q.s
Pineapple flavor*	Water	Water	Water	Water	Fractionated	Refind
Suspending medium*	q.s	q.s	q.s	q.s	Coconut oil q.s	Almond oil q.s
pH	5	5.5	6	6.5	-	-
Citric acid	q.s	q.s	q.s	q.s	-	-

* Quantity sufficient

Table 2: Comparative evaluation of Ampicillin trihydrate suspension formulations

Parameters Evaluated	**F-I**	**F-II**	**F-III**	**F-IV**	**F-V**	**F-VI**
Appearance	Paleyellow	Paleyellow	Paleyellow	Paleyellow	Paleyellow	Paleyellow
Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
Viscosity (mps100rpm)	373	320	315	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
Particlesize range (μm)	15-250	15-250	15-250	15-250	15-250	15-250
Drug content (%)	104±0.12	104±0.09	102.1±0.08	104.1±0.04	104.2±0.04	102.2±0.09
In-vitro % drug release(After 50 mins)	100.55	100.76	100.49	100.96	100.67	100.25
Rf Value	0.95	0.95	0.95	0.95	0.95	0.95

Accelerated Stability Studies at 40^{0}C/ 75% RH

Assay for content uniformity

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**
1st day	25mg/ml	104±0.08	104.2±0.08	104.3±0.04
7th day		98.1±0.16	99.1±0.08	96.8±0.08
14th 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**
1st day	25mg/ml	102.3±0.09	102.4±0.04	104±0.08
7th day		97.6±0.12	96.5±0.16	97.33±0.12
14th day		59.9±0.12	68.6±0.87	76.1±0.04

* Standard deviation N=3

Ease of redispersibility[8]

The suspension was allowed to settle in a measuring cylinder. The mouth of the cylinder was closed and was inverted through 180^0 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 redispersable. 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 100rpm.The temperature was maintained at 30^{o}C.

Particle size distribution [9, 10]

Using optical microscope carried out particle size distribution studies.

1. Eye piece micrometer was calibrated using stage micrometer, X-number of eyepiece divisions=y number of stage micrometer divisions.
2. Eye piece division= (Y least count/X)
3. Sample was uniformly suspended in paraffin oil.
4. 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 U.S.P (XXII) rotating paddle dissolution apparatus (apparatus II). The dosage forms were placed in 900ml of distilled water as a medium at 37^0(± 1^0 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^{0}C/60%RH and 30^{0}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 calculations13.

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 Rf values were calculated for standard and sample.

RESULTS AND DISCUSSION

Physical characteristics of readiuse oral suspension of Ampicillin were measured. According to methods describe above.

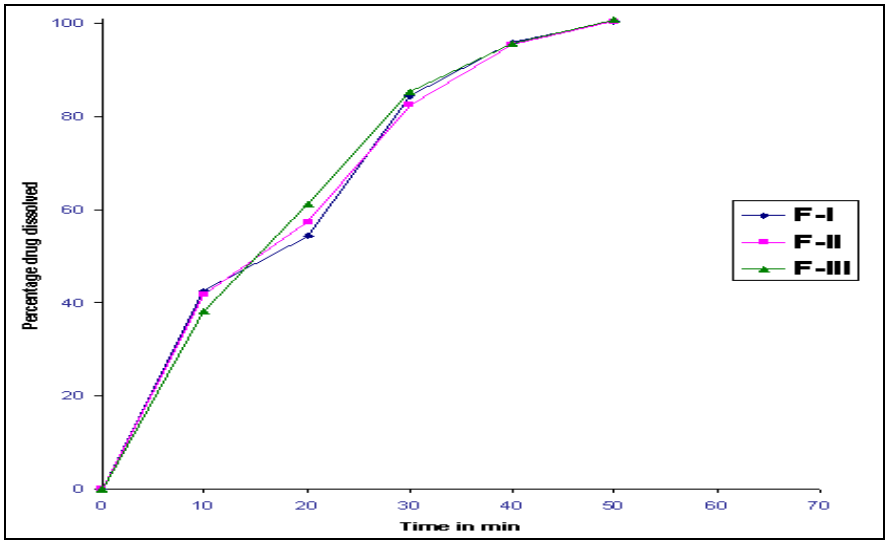

Fig. 1: Percentage of drug dissolved vs time for formulation F-I, F-II and FIII

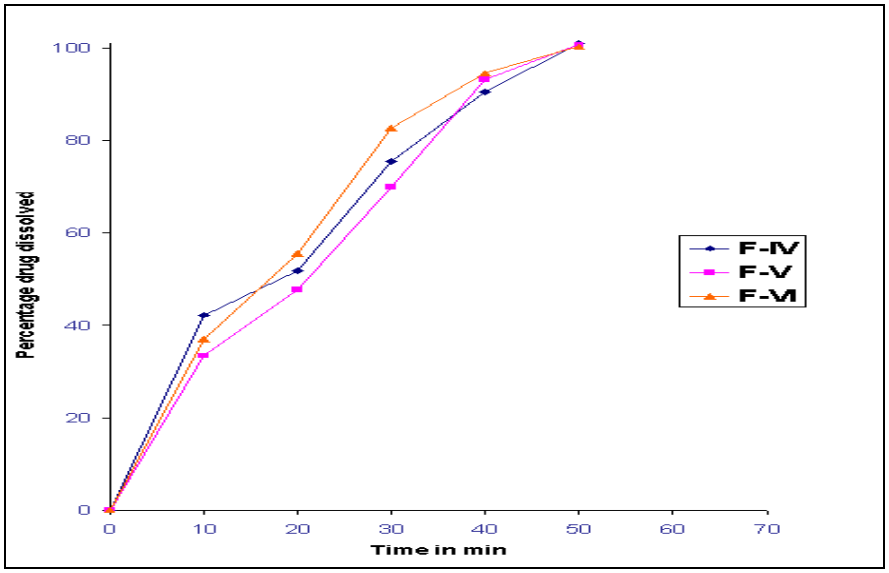

Fig. 2: Percentage of drug dissolved vs time for formulation F-IV, F-V and F-VI

Viscosity is an important parameter for characterizing the suspensions as it affects redispersibility ,pouring from container and drug release. All the formulations was such that it would be easily pourable from container and also showed a shear thinning effect.

The redispersibility of all prepared formulation were found to poses an excellent redispersibility property with optimum particle size distribution .Sedimentation volume of all formulation is below 1, which in indicates that the formulations were of optimum and

acceptable. All the prepared formulations showed uniformity in drug content and were within permissible range of standard limits of the pharmacopoeia.

The results of the comparative evaluation of physical characteristics of Ampicillin trihydrate rediuse oral suspensions are shown in table - 2.

The prepared ready mix formulations of Ampicillin trihydrate were found to process 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. In-vitro dissolution studies of the prepared formulation proved that Ampicillin trihydrate release for all the formulations was almost similar with 100%dissolution within 50minutes (fig1&2)

Data in table 3(a) and 3(b) shows that the drug is not stable at 40^0C and 75% RH. As it is clear from the table that within 15 days the drug is degraded below the pharmacopoeia limits. According to the ICH- guidelines[15] 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 40^0C / 75%RH, then stability testing is to be carried out at 30^0C /60% RH. So testing was carried out at 30^0C /60% RH and an additional study was carried out at 25^0C /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 70^{th} (95.7%±0.12 & 96.2%±0.04) and 77^{th} (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, .From above results it can be concluded that Ampicillin trihydrate can be formulated as rediuse oral suspension with improved shelf life.

REFERENCES

1. Ansel, H.C., Eds.In: Introduction to Pharmaceutical Dosage Forms, 2^{nd} Edn. 95- 98.
2. Martin,A,et al ,Eds.In:Physical Pharmacy,4^{th} Edn,477.
3. Ansel, H.C., Eds.In: Introduction to Pharmaceutical Dosage Forms, 2^{nd} Edn. 142-144.
4. Deorge, R.D., Eds.In: Wilson and Gisvold Text Book of Organic Medicinal And Pharmaceutical Chemistry, J.B.Lippincoot Co.U.S.A, 8^{th} Edn. 241.
5. Kohli, D.P.S., Eds. In: Drug Formulation Manual, 542-543.
6. Klaus Florey. Eds.In: Analytical Profiles of Drug substances, Vol-II, 38-34.
7. Banker.G. S., Eds.In: Modern pharmaceutics, Marcel Dekker Inc., Vol 7, 347-349.
8. Elkheshen, S. A., Optimization of a Reconstitutable Suspension of Rifampicin Using 2^4 Factorial Designs, Drug Dev. Ind. Pharm, 1996, 22(7), 623-630.
9. Eugene, L. P., Eds.In: Experimental Pharmaceutics, 4^{th} Edn., 21.
10. Martin, A., *Eds*.In: Physical Pharmacy, 4^{th} Edn. 426-430.
11. Wagh, A., In-*vitro* Evaluation of Commercial Ampicillin Capsules, The Eastern Pharmacist, October 1995, Vol.37 (454), 145-147.
12. Indian Pharmacopoeia. The controller of publication, Delhi, 1996, Vol.-I, 55.
13. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use,ICH-3,chapter 9,ICH-Technical co-ordination, London.