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FORMULATION AND EVALUATION OF CEPHALEXIN MONOHYDRATE RECONSTITUTIONAL ORAL SUSPENSION WITH PIPERINE AND THEIR ANTIBACTERIALACTIVITY

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ABSTRACT

The modern scientific and technological advancement in the pharmaceutical field had created bank of interest in reconstitutable oral suspension dosage form in the recent year. A variety of technical and scientific approaches is been conducted in the field of research and development for the formulation of reconstitutable oral suspension. By addition of piperine in reconstitutable oral suspension of cephalexin monohydrate, increase the bioavilibility of dosage form so its antibacterial activity is increase. The basic aim behind developing this reconstitutional oral suspension system was to improve the chemical stability, increase the bioavailability and controlled the duration and onset of action of the drug. The purpose of writing this thesis work on reconstitutable oral suspension of cephalexin monohydrate was to collect all the recent updates from the literature and do on this basis with the main goal to method of prepration of the reconstitution oral suspension, good qualities of ideal reconstitutional oral suspension, factors to be considerd at time of prepration and its evalution parameter.

KEY WORDS: piperine, suspension, cephalexin.

INTRODUCTION

Since decades among all the pharmaceutical products available,oral drug delivery had gained a higher scope and popularity and had been widely employed for the systemic delivery of drug.the positive aspect regarding the oral dosage form which created its high level of acceptance was its case of administration,patient compliance and stability in the formulation[1]

Dry Powder Of Oral Reconstitutable Suspension

Dry powder reconstitutable oral suspension can be administere immediately. There are an important category of suspension that requires mixing prior to administration. These are dry mixtures that require the addition of water at the time of dispensing. The reconstituted system is the formulation of choice when the drug stability is a major concern. After reconstitution, these systems have a short but acceptable life if stored at refrigerator temperatures[2,3].Reconstitutable oral systems show adequate chemical stability of the drug during shelf life, avoids the physical stability problems related to solubility, pH,and incompatibilities with other ingredients and also reduce the weight of the final product because the aqueous vehicle is absent and consequently the transportation expenses may be reduced[4].the medication are supplied in dry form because the product can be stored for a long time in dry form but become deteriorate in solution within a relatively short time.such solution are said to have a short shelf life.[5]A reconstitutable suspension can offer several advantages such as maintenance of the chemical stability of the active compounds until reconstitution at the start of treatment. The same suspension can be easily administered to children of different ages by adapting the volume to swallow[6].

QUALITIES OF IDEAL ORAL SUSPENSION[7]

(1)The dispersed particle should not settle readiy and the settle should redispersed immediately.

(2)The particle should not form a cake on settling.

(3)The viscosity should be such that the prepration can be easily poured.

(4)It should be chemically and physically stable.

(5)It should be palatable.

(6)It should be free from gritting particle.

ADVANTAGES[8]

1. Pharmaceutical oral suspension can improve chemical stability of certain drug.
2. Drug in oral suspension exhibit higher rate of bioavailability than other dosage form.

3. Duration and onset of action can be controlled.
4. Oral suspension can mask the unpleasant bitter taste of drug.

MATERIAL ND METHOD

Material

Cephalexin monohydrate was obtained as gift sample from Innova Captab Pvt. Ltd. Sodium citrate,Citric acid and Sodium benzoate were obtained from the Merc Limited. All other ingredients were used of pharmaceutical grade. Piperine is extracted and isolated in laboratory from black pepper[9].

Method: Isolation of piperine[10,11]

Take 150 ml of 95% ethanol in round bottom flask and 5-10 boiling chips added. Take 15 gm of black pepper powder in the soxhlet apparatus and heat the reflux for 2-2.5 hours. Then the extracted mixture of round bottom flask was filtered by using suction pump. Then concentrate the filtered solution to a volume of 15-20 ml by simple distillation. Took the concentrated pepper extract in 125 ml of Erlenmayer flask and add the 10 ml of 10% solution of KOH and heated. The refluxing mixture was diluted with addition of water till the precipitation formation stopped. A brownish yellow precipitate was formed. The precipitate mixture is allowed to stand overnight. The resulted extracts were collected by filtering the precipitate by suction pump and then re-crystallized by 10-20 ml of acetone. A yellowish white crystal of piperine was obtained.

PREFORMULATION STUDIES[13,14,15]

All the excipients and drug were passed through sieve No. 60. The weight quantity of each ingredient was sized to a required degree of fineness (except talc and magnesium stearate). The powder blend was evaluated for the fallowing properties as fallow:-

Angle of repose

Angle of repose was determined by using the fixed funnel method. The blend powder was poured through a funnel that may be raised vertically maximum cone height (h) was obtained. Radius (r) of the heap or pile was measured and the angle of repose (q) was calculated by using this formula:

Tan q = h/r

q = tan-1 h/r

Table 1: Angle of Repose as an Indication of Powder Flow Properties

Sr.No.	Angle of Repose(^{o})	Type of flow
1	<20	Excellent
2	20-30	Good
3	30-40	Passable
4	> 44	Very poor

Bulk density

The bulk density (Db) was determined pouring the blend material into a graduated cylinder. The bulk volume (Vb) and weight of the blend (M) was material noted. The bulk density was calculated by using the fallowing formula:

Db = M/ Vb

Where, M is the mass of blend powder Vb is the bulk volume of the lend powder.

Tapped density

The known mass (M) of the blend material was placed on the measuring cylinder and was tapped for the fixed number of times. The minimum volume (tapped volume Vt) occupied in the cylinder. Tapped density (Dt) was calculated by using the fallowing formula:

Dt = M / Vt

Where, M is the mass of powder Vt is the tapped volume of the powder.

Carr's index or compressibility index

This is the simplest way of measuring the free flowing property of the powder. It is expressed in the percentage (%) and can be calculated b the fallowing formula:

$$\text{Compressibility Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Table 2-Relation between % Compressibility and Flowability

% Compressibility	Flowability
5-15	Excellent
12-16	Good
18-21	Fair passable
23-35	Poor
33-38	Very Poor
>40	Very Very Poor

Formation of reconstitutional oral suspension by powder mixture method[16]

1-Powder mixture of cephalexin(dose 125\5ml) was prepared using suspending agents, swetner, preservative, buffer, flavourant and anticacking agent by conventional technique.

2-All ingradients were passed through 200# and then mixed by geometric dilution.

3-Dose of powder mixture 3.4gm of powder to 5ml equivalent to 0.125gm of cephalexin.

4-Add pipperine extract with powder mixture at amount 10mg\200mg of powder and mixed all ingradients.

5-Add distilled water for making suspension form.

EVALUATION OF RECONSTITUTIONAL ORAL SUSPENSION[17,18]

Drug content

One dose (3.4 g of the formulation to 5 ml) is equivalent to 0.125g of Cephalexin. The drug was extracted with 100 ml of distilled water and the solution was filtered through nylon filter membrane (0.22 µm). 0.1 ml of the solution was further diluted to 10 ml with distilled water and absorbance of the solution was read at λmax 260 nm on Hitachi U-2800 UV spectrophotometer. The drug concentration was extrapolated from the calibration curve in distilled water.

PH

The pH of reconstituted suspension was determined using a pH meter-Systronic µ pH system 361. A glass rod was dipped into suspension containing 100 mg of drug filled in a 50 ml of beaker.

Viscosity

The rheologic parameters of the prepared suspensions, in terms of viscosity, were determined by use of the steady shear method, measuring the “non-Newtonian viscosity” . Rheology of all suspensions was performed with a RVT Brookfield viscometer from Choksi Lab. (Indore, M. P.) All measurements were performed after eliminating all thixotropy from the suspension.

Sedimentation volume ratio

To study sedimentation of our suspensions, the sedimentation volume was determined as a function of time. The sedimentation volume F is defined as the ratio of the final, equilibrium volume of the sediment, Vu to the total volume V0 before settling, as expressed in the following equation:

F = [Vu/V0].

In this study, the sedimentation volume was determined as a function of time. The suspension (height = 9 cm) was decanted in a cylinder of 100 ml with a diameter of 2.5 cm. After 1 h, 24 h and 1 week, the sedimentation volume F was determined.

Invitro drug release

The *in vitro* dissolution studies were carried out using USP apparatus Type II at 100 rpm. The dissolution medium consisted of 900 ml distilled water maintained at 370C ± 0.50C The drug release at different time intervals was measured for two hours using Hitachi U-2800 UV spectrophotometer.

EVALUATION OF ANTIBACTERIAL ACTIVITY OF FORMULATED RECONSTITUTIONAL ORAL SUSPENSION[19,20]

Zone of Inhibition

1. **Method- Disc Diffusion Method**
2. **Media** – Muller Hinton Agar
3. **Species**- *A) Gram Positive- S. Aureus*

B) Gram Negative- E. coli

4. **Solvent**- DMSO
5. **Concentration** – 100 µg/Ml

Minimum Inhibition concentration

6. **Method- Broth Dilution Method**
7. **Media** – Muller Hinton Broth
8. **Species**- A) Gram Positive- Staphylococcus aureus

B) Gram Negative- E. coli

Solvent- DMSO

Table 3-Composition of cephalexin monohydrate with pipperine suspension

Formulations	F-1(gm)	F-2(gm)	F-3(gm)	F-4(gm)	F-5(gm)	F-6(gm)
Cephalexin Monohydrate	0.425	0.425	0.425	0.425	0.425	0.425
Pipperine extract	0.01	0.01	0.01	0.015	0.015	0.015
Sucrose	10.625	10.625	10.625	10.625	10.625	10.625
Tragacanth	0.17	_	_	0.17	_	_
Acacia	_	0.17	_	_	0.17	_

Polyvinyl pyrrolidine	_	_	0.17	_	_	0.17
Sodium citrate	0.034	0.034	0.034	0.034	0.034	0.034
Citric acid	0.068	0.068	0.068	0.068	0.068	0.068
Sodium benzoate	0.034	0.034	0.034	0.034	0.034	0.034
Aerosil	0.85	0.85	0.85	0.85	0.85	0.85
Sunset yellow	q.s	q.s	q.s	q.s	q.s	q.s
Lemon flavour	q.s	q.s	q.s	q.s	q.s	q.s

Table 4- Evalution of powder mixture

	Angle of repose	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr's index (%)
F1	12.5± 0.02	0.625	0.8± 0.01	1.9
F2	12.5± 0.12	0.66± 0.01	0.80	2.5
F3	16.75± 0.01	0.625± 0.04	0.833± 0.01	8.26
F4	13.7± 0.02	0.633± 0.01	0.822± 0.01	5.19
F5	12.5± 0.12	0.615± 0.03	0.8± 0.02	3.125
F6	14.5± 0.02	0.65± 0.04	0.823± 0.02	3.98

Table 5- Evaluation data for drug content and organolepticproperties

Frmulation	Drug content(%)	Organoleptic properties			
		Colour	Odour	Taste	Appearance
F1	99.0± 0.4	Yellow	Lemon	Palatable	Smooth
F2	99.8± 0.2	Yellow	Lemon	Palatable	Smooth
F3	99.9± 0.1	Yellow	Lemon	Palatable	Smooth
F4	98.9± 0.4	Yellow	Lemon	Palatable	Smooth
F5	99.9± 0.2	Yellow	Lemon	Palatable	Smooth
F6	98.0± 0.2	Yellow	Lemon	Palatable	Smooth

Table 6- Evaluation data for PH and Viscosity

Formulation	PH	Viscosity(cps)
F1	6.0	95.0± 0.4
F2	6.0	95.8± 0.2
F3	6.0	96.8± 0.5
F4	6.0	95.0± 0.12
F5	6.0	98.7± 0.55
F5	6.0	98.2± 0.22

Table 7-Evaluation data for Sedimentation volume ratio

Formulation	E-1(h)	E-24(h)	E-72(h)	E-168(h)
F-1	0.13	0.28	0.26	0.24
F-2	0.52	0.30	0.28	0.26
F-3	0.54	0.34	0.31	0.29
F-4	0.06	0.38	0.36	0.34
F-5	0.85	0.80	0.75	0.72
F-6	0.92	0.86	0.84	0.84

Table 8-Evaluation data for Invitro drug release

Time(min)	F-1	F-2	F-3	F-4	F-5	F-6
10	42.20	40.38	38.21	43.72	38.54	37.92
20	53.21	56.71	59.06	51.72	47.34	57.53
30	82.32	80.23	83.12	75.32	69.82	81.72
40	92.30	93.62	91.74	88.32	89.52	90.37
50	99.56	99.72	99.31	98.75	99.52	99.73

Table 9-Rate of zone of inhibition of S. aureus and E. coli by disk diffusion method.

Sr. No.	Comp. Code	*S. aureus*	E. coli
		ZONE OF INHIBITION (In mm)	
1	**F1**	14	03
2	**F2**	16	04
3	**F3**	15	03
4	**F4**	16	04
5	**F5**	10	01
6	**F6**	11	01
	Std	**Vancomycine**	**Amikacine**
		08	**20**

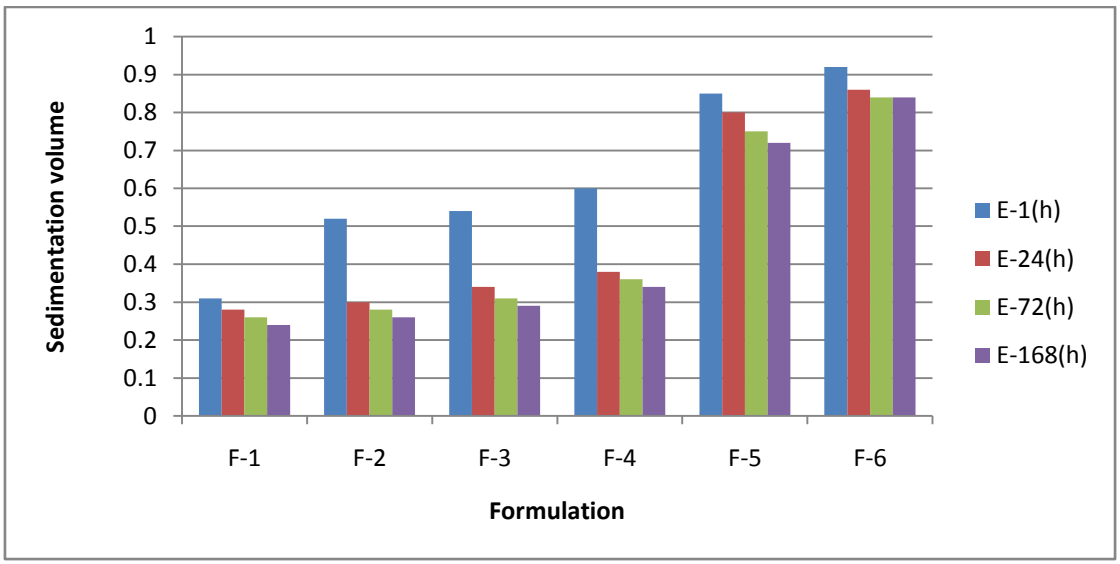

Figure 1- Sedimentation volume of cephalexin monohydrate reconstitutional oral suspension

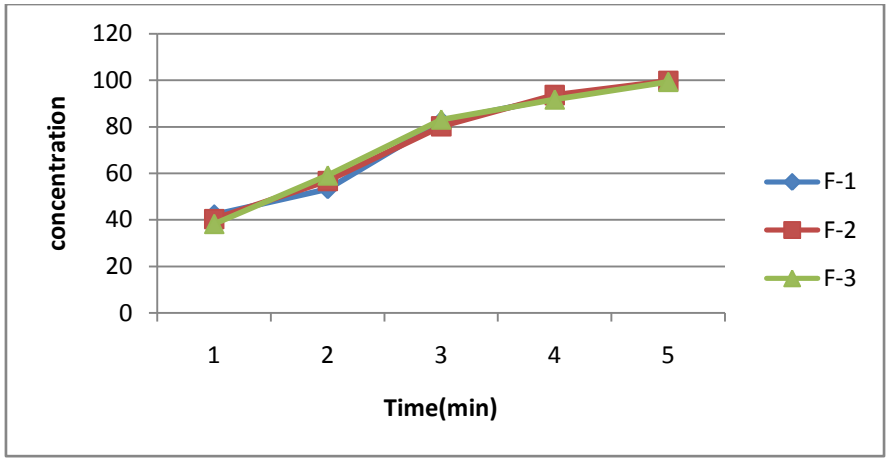

Figure 2- Invitro drug release of formulation F-1,F-2 and F-3

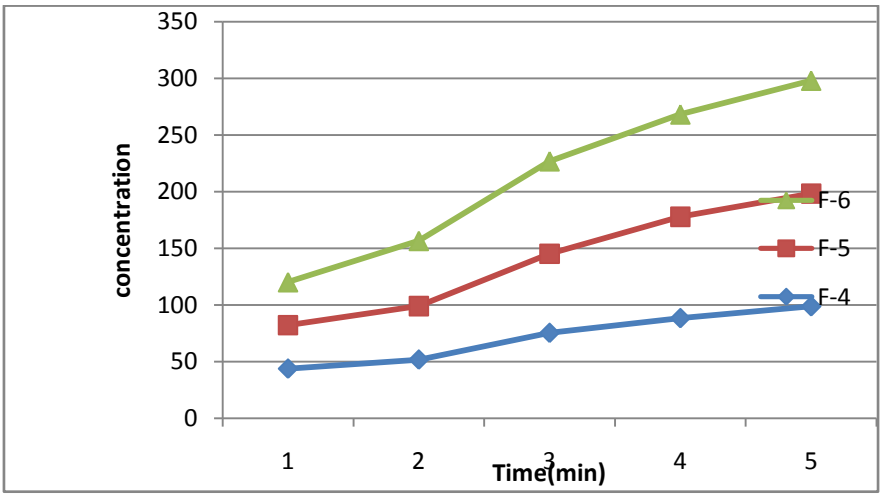

Figure 3-Invitro drug release of formulation F-4,F-5 and F-6

CONCLUSION

Six batches have been formulated of cephalexin monohydrate with combination of piperine and excipient for each formulation drug and all excipient with piperine were prepared and evaluated by various parameter.the angle of repose of F-3 was in the 16.75± 0.01, the drug content of F-1 was in the range 99.0±0.4,the viscosity of formulation F-5 was in the range 98.7±0.55,the sedimentation range of formulation F-3 was in the 0.29 and Invitro drug release of formulation F-5 is 99.52 Zone of inhibition of S.aureus and E.coli are tabulated in the table no:9 formulation F-2 and F-4 show best result.

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