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DEVELOPMENT OF DICLOFENAC SUSPENSION AND ITS STABILITY STUDY AT DIFFERENT TEMPERATURES

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ABSTRACT

Diclofenac is the most widely used non-steroidal anti-inflammatory drug. However, its oral aqueous formulation is not available in market because of its bitter taste and scratching sensation in throat. This study was conducted to develop a stable suspension formulation of diclofenac. Diclofenac acid suspension was prepared by making some modifications in the previously described method. .For stability study, the suspension was placed in twelve amber colored divided in four sets each consisting of three bottles. The three sets were stored at 4ºC, room temperature (22ºC±5ºC), 40ºC and 60ºC. The contents of drug in suspension were analyzed immediately after preparation (at zero time), after 2 h, 4 h, 6 hours, 12 h, 24 h, 48 h at 7 days and thereafter, at the end of every week until 13 weeks. To measure drug in sample, diclofenac acid suspension was diluted in absolute ethyl alcohol to make 8ppm of the drug. This solution was filtered and absorbance was measured at 284nm using absolute ethyl alcohol as the blank solution on UV spectrophotometer. The stability was assessed as %age remaining of active content. The highest % remaining drug, 96.03% and 94.93% was found in suspension stored at 4ºC and at room temperature, respectively. The shelf life for suspension stored at 4ºC, room temperature, 40ºC and 60ºC was 250 days, 200 days, 66.6 days and 62.5 days, respectively. The physical parameters such as: taste, odor, color, visual appearance, sedimentation volume, Re-dispersion, rheological Properties, and pH were also regularly checked and monitored at the end of every week up to 13 weeks.

Keywords: Diclofenac acid, Stability, Suspension, Temperature.

INTRODUCTION

Diclofenac is a potent analgesic and anti-inflammatory drug. It acts by reducing the release of arachadonic acid and enhancement of arachadonic acid uptake. So, it has a dual inhibitory effect on both the cyclo-oxygenase and lipo-oxygenase channels (Scholer *et al.,* 1986). Diclofenac exhibits fewer side effects is believed to be better tolerated as compared to other anti-inflammatory drugs. Among the 20% patients who are on long-term therapy of diclofenac, only 2% have to discontinue because of its gastrointestinal side effects (Dhikav *et al.,* 2002). Dosage forms for diclofenac are available for oral, rectal and intramuscular administration. Dosage adjustment is not required usually except in case of elderly patients, or in patients having hepatic or renal impairment. Diclofenac has a shorter half life which limits the possibility of its accumulation in body. It has a quick onset of action and long duration of therapeutic efficacy. When diclofenac is administered intra-muscularly, it is almost equal and sometimes even superior to many narcotic and spasmolytic drugs in biliary and renal colic (Todd and Sorkin, 1988). Most common side effects of non-specific non-steroidal anti-inflammatory drugs (NSAIDs) are: upper gastrointestinal hemorrhage, nausea, vomiting, perforation, bleeding,

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pain, dyspepsia, heartburn, and ulceration leading to death (Hawkey *et al.,* 1991). It has been reported that in users of conventional NSAIDs the prevalence rate of gastric and duodenal ulcer is 14-25%. Another common side effect of NSAIDs is inflammation of small intestine along with blood loss and protein loss. Because of this blood loss and protein loss, the patient may suffer from iron deficiency anemia or hypoalumina emia (Graham *et al.,* 1988).

MATERIALS AND METHODS

Drug and chemicals

Following drug and chemical were used in this study: Diclofenac acid 99.6% purity (Sami Pharmaceuticals (PVT) Ltd. Karachi), Ethyl alcohol absolute (RDH Laborchemi Kalien GmbH & Company), Sorbitol liquid USP, Sorbic Acid extra pure, Hydroxy ethyl cellulose Sodium Saccharine dehydrate Ascorbic Acid BP, USP Sodium Chloride Potassium Chloride (E. Merck, Germany), Disodium hydrogen phosphate Formalin 10% solution), (E. Merck, Germany), Banana Aroma Syrup (West Brook Corporation, USA), Golden Invert Syrup (Corn Products International, USA), Microcrystalline Avicel® (Barcelona, USA), Deionized water (Medilines Diagnostic division, Hematoxylin), Eosin Dye (BDH Labs, England).

Preparation of diclofenac acid suspension

One percent by weight of diclofenac acid suspension was prepared after some modifications in the method described by Affolter and Heidi (1992) using the ingredients given in Table I.

Table I: Ingredients and proportions of diclofenac acid suspension

No.	Ingredients	Proportion (g)
1	Diclofenac (free acid)	1.00
2	Aroma golden syrup	0.60
3	Avicel®	1.20
4	Banana aroma	0.05
5	Citric acid	0.80
6	Hydroxy ethyl cellulose	0.50
7	Saccharine sodium	0.10
8	Sorbic acid	0.10
9	Sorbitol solution	27.0
10	Vitamin. C	0.60
11	Deionized water	76.05

Avicel® was suspended in water by using a high speed mixer. Hydroxy ethyl cellulose was admixed in the above mixer and the mixture was left to swell for about one hour. Sorbitol liquid (USP) and sorbic acid were then added and the mixture was heated on a hot plate to about 85ºC with continuous stirring. After the mixture has cooled to room temperature, vitamin C, citric acid and Aroma Golden Syrup® were added and dissolved consecutively by stirring (Magnetic Stirrer / Hot Plate (Germany). Finally diclofenac Acid was added and suspended therein using the hot / stirring plate. Afterwards, the formulation was put in a conical flask and shaken in Orbital Shaking Incubator (Pamico Equipments, PA -42 / 250 R, Faisalabad) for 10 min.

Spectrophotometric assay of diclofenac acid suspension

It was measured that each 0.1ml of diclofenac acid suspension contains 1mg of diclofenac acid.

Preparation of standard solution

One milligram of diclofenac acid (99.66% pure) was dissolved in 125ml of absolute ethyl alcohol to get a clear, transparent solution of 8ppm strength. The solution was filtered and 15ml of it was taken to be used as a standard solution in UV spectrophotometer.

Preparation of sample solution

An 8ppm dilution of diclofenac acid suspension was made by dissolving 0.1ml of diclofenac acid suspension (1mg of diclofenac acid) in 125ml of absolute ethyl alcohol. This solution was then filtered and 15ml of it was taken in a test tube. The absorbance was measured at 284nm using absolute ethyl alcohol as the blank solution on UV spectrophotometer.

Evaluation of taste acceptability of diclofenac acid suspension

Since diclofenac acid has a bitter taste and causes scratching sensation in throat, therefore the taste of diclofenac acid suspension was improved by using different excipients as sweetening agents. Six different formulations were made by using different concentrations of sweetening agents in each formulation (Srisagul, 2004) as shown in Table II. The suspension formulations of diclofenac acid were prepared by procedure given above.

Taste acceptability of diclofenac acid suspension was assessed by using a flavor panel consisted of 20 volunteers from the members of pharmacy teaching faculty and the students from Department of Pharmacy, University of Sargodha. The panel members were briefed about the nature and purpose of this evaluation study. Each volunteer was instructed to:

A. take half spoonful of the formulation.
B. record the score of the rating of formulation on the evaluation sheet.
C. wait for 10 minutes before tasting the next formulation so that the taste of first formulation was subsided.

Table II: Diclofenac acid suspension formulations designed for taste acceptability evaluation.

No.	Ingredients	Formulations					
		1	2	3	4	5	6
1	Diclofenac (free acid)	1.00	1.00	1.00	1.00	1.00	1.00
2	Aroma golden syrup	1.20	1.20	1.20	1.20	1.20	1.20
3	Avicel®	0.500	0.500	0.500	0.500	0.500	0.500
4	Banana aroma	0.600	0.7000	0.800	0.600	0.700	0.800
5	Citric acid	0.300	0.200	0.100	0.300	0.200	0.100
6	Hydroxy ethyl cellulose	0.600	0.600	0.6000	0.600	0.600	0.600
7	Saccharine sodium	20.00	25.00	27.00	20.00	25.00	27.00
8	Sorbic acid	0.400	0.500	0.600	-	-	0.800
9	Sorbitol solution	0.050	0.050	0.050	0.050	0.050	0.050
10	Vitamin. C	0.080	0.090	0.100	0.080	0.090	-
11	De-ionized water	70.00	73.00	76.00	70.00	73.00	76.00

D. rate each formulation from 1 to 10; 1 being the lowest score depicting the most disliked taste and 10 being the highest score depicting the most liked taste of the formulation.

The formulation that earned the highest score in taste evaluation study was chosen for further study (Srisagul, 2004).

***In-vitro* stability studies**

The *in-vitro* stability was studied for the suspension which achieved the highest taste acceptability score (Table III).The stability study was carried out by putting the suspension in twelve amber colored glass bottles. These twelve bottles were divided in four sets, each consisting of three bottles and were stored at 4ºC, Room temperature (22ºC±5ºC), 40ºC and 60ºC. The suspension was analyzed by UV spectrophotometer, immediately after preparation (at zero time), after 2 h, 4 h, 6 h, 12 h, 24 h, 48 h, 7 days and thereafter, at the end of every week until 13 weeks (Alexander and Thyangarajapuram, 2003). The active content in suspension formulations was checked by measuring the absorbance of sample solutions on UV spectrophotometer at 284nm wavelength at above mentioned time intervals and by calculating the %age remaining of active content by following formula (USP, 2003):

$$\%\ \text{Content in sample} = A \times B \times C$$

Where

$$A = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}}$$

$$B = \frac{\text{Concentration of Standard}}{\text{Concentration of Sample}}$$

$$C = \%\ \text{Purity of Standard}$$

Physical parameters of diclofenac acid suspension

Following physical parameters were studied at the time of preparation of suspension and later at the end of every week until 13 weeks: visual appearance, odor, taste, sedimentation volume, re-dispersion, density, viscosity and pH.

RESULTS AND DISCUSSION

A suspension of diclofenac acid was prepared in six formulations using the different concentrations of sweetening agents. The formulation no. 3 (shown in Table III) was the most highly rated by the volunteers and it was used for the further stability studies. Accelerated stability testing was performed at 4ºC, room temperature, 40ºC and 60ºC for 13 weeks (91 days). The % drug remaining in standard and test formulation at zero time and at the end of 13 weeks is shown in Figure 1. The above and the other parameters for test suspension are shown in Table IV and Figure 1.

Table III: Outcome of taste acceptability evaluation test

Formulation	Mean rating
1	7.60 ± 1.44
2	6.92 ± 1.44
3	7.94 ± 1.33
4	6.88 ± 1.42
5	6.84 ± 1.31
6	6.62 ± 1.63

The above results showed that the suspension formulation of diclofenac acid remained stable at 4ºC, as the %age of drug remaining was not decreased more than 5% (Remington, 2000). The suspension formulation of diclofenac acid stored at room temp. (22 ± 5ºC) was also found to be very close to the standard which was set for the remaining %age of drug content in pharmaceutical

Table IV: Different parameters studied for the selected diclofenac acid suspension formulations at different temperatures

Time	4ºC	Room Temp	40ºC	60ºC
Purity (%) at day 0	-	99.34 ± 0.632	-	-
Purity (%) at 13 weeks	96.03	94.93 ± 0.618	89.58	85.17 ± 0.587
Shelf life (days)	250	200	66.6	62.5
Density	1.097	1.087	1.081	1.070
Viscosity	151.25	145.71	140.76	125.03

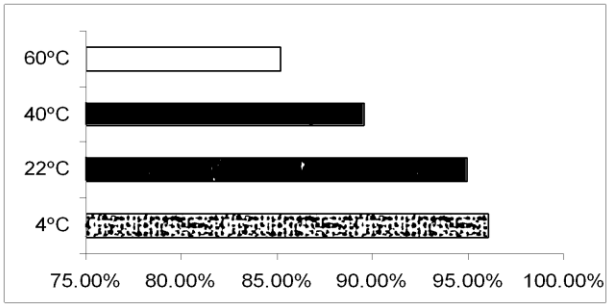

Figure 1: Stability of the selected diclofenac acid suspension formulation after 13 Weeks

suspensions during accelerated stability studies (William, 1997).

The shelf-life of suspension stored at different temperatures were calculated and given in Table IV. As one of primary objectives for developing suspension formulation of diclofenac acid was taste masking, therefore the taste was regularly checked at the end of every week until 13 weeks and it was found that the suspension formulations stored at 4ºC and at room temperature were best in taste while that stored at 40ºC was slightly bitter but still palatable in taste however the suspension formulation stored at 60ºC was not palatable after duration of 13 weeks. Taste of a pharmaceutical suspension depends mainly on the suspending agents and the sweetening agents used (Hashem and Ramden (1987). Visual appearance and odor were best in the formulation stored at 4ºC and room temperature. The pH values were surprisingly not found different at all temperatures for a period of three months.

The density, an important parameter for suspension stability was found at the end of three months for suspension formulation and is given in Table IV. It is evident from the above results that the density decreased with rise in storage temperatures at the end of 13 weeks, which shows that to keep the stability of diclofenac acid suspension, it is necessary to maintain the density along with the passage of time (Sharma, 2005). The same concept goes with the viscosity of pharmaceutical suspensions as density and viscosity are inter-related (Willings *et al.,* 2007). The viscosity of diclofenac acid suspension at different temperatures at the end of 13 weeks is also given Table IV.

It is evident from the above findings that the viscosity remained maximally stable at 4ºC and at room temperature which made the suspension more stable by making the degradation process slower (Liebermann *et al.,* 2008).

Another physical property which determines the stability of pharmaceutical suspensions is the sedimentation volume (Martin, 2001). Sedimentation volume was found to be constant at the end of 45 days at room temperature, which means the overall consistency and the physical form of diclofenac acid suspension was not changed and did not form hard cake on storage (Swarbrick *et al.,* 2000). Re-dispersion was also done easily when the suspension was shaken vigorously at many time intervals until 13 weeks.

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