

## ANNATTO SEED COLOR AS NATURAL COLORING AGENT IN ORAL DOSAGE FORMS

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### Abstract:

Pharmaceutical excipients are the backbones of pharmaceutical industries. All most all dosage forms need various types of excipients for their formulations, one such excipient is coloring agent. Annatto seed color is widely used as natural food color, but it has never been used as coloring agent in pharmaceutical dosage forms. Here we established new methods and techniques to process the annatto seed colors to be used as pharmaceutical coloring agents. In the present experiment an effort has been made to study on annatto seeds to utilize as coloring agents in oral liquid and solid dosage forms. The color was extracted from the seeds by different methods. The characterization of the extracts was done by UV-Vis spectrophotometric method, FTIR method with or without using different excipients and drugs. The comparative study of the annatto seed extract with standard methyl orange has been done and the stability study of the color in oral liquid dosage form (i.e. in simple syrup), as well as in solid dosage form (i.e. in tablets) has been done to know the physical and chemical integrity. The toxicity study of the annatto seed extract was done in Albino Rat by oral administration. The color intensity of annatto seed extract was found to be more than that of the methyl orange at same ppm strength and stable in liquid and solid oral formulations. The colors are nontoxic, biodegradable and economically viable color supplement for oral liquid and solid dosage forms.

**Key words:** Annatto seed, color, liquid oral, tablet, stability and toxicity.

### Introduction:

Oral dosage forms are most convenient dosage forms among all, intended for systemic effects (Aulton, 2002) they include tablets, capsules, syrups, solutions, emulsions and suspensions etc (Martin, 2006). Coloring agents are used to impart a distinctive appearance to pharmaceutical dosage forms to make it attractive to the patients (Hess, et al., 1979 and Code of Federal Regulation, 2002). The widespread and relatively large use of colors in food, a number of coloring agents in current use have been associated with adverse effect (Winter, et al., 1989). In general the safety of coloring agents in pharmaceutical and foods are associated with report of hyper sensitivity (Bell, 1991, Levesque, et al. 1991 and Dietemann, et al., 1991) and hyper kinetic activity especially among children (Pollock, et al., 1989). The addition of coloring matters to medicinal products governs primary legislations. Annatto (*Bixa Orellana*) is an evergreen and small tree available in tropical regions. The seed is valued as source of pigment, which impart red or orange hue (Kalsec, 2002). Its coloring properties are mainly attributed to its constituents, carotenoid, bixin and nor-bixin, which comprise 70 to 80% of the total pigment mass surrounding each individual annatto seed (Minnguez-Mosquera, et al., 1995). The alkali extract is found to be water soluble and stable at pH 8 and used in different fruit and vegetable products (Satyanarayana, et al., 2006). An attempt has been made to characterize the color extracted from annatto seed and standardize against methyl orange by UV-Vis spectrophotometric method. The physical and chemical stability of color intensity with simple syrup (Remington's, 2006) at different concentration of annatto seed extract at different temperature both at ambient as well as accelerated conditions were done to know the chemical integrity (Remington's, 2006, Leon Lachman, et al., 1987, Garret, et al., 1955, Martin, 2006 and John, 2000). Uncoated tablets of diclofenac sodium containing 50mg of diclofenac sodium each were prepared and colored film coated with different concentrations (0.05, 0.1, 0.15% w/v solutions) of annatto seed extract powder. The stability studies

in accelerated conditions (Saha, et al, 2007) as well as other physico-chemical parameters of uncoated as well as colored coated tablets (Vijayalaxmi, et al., 2004) were studied to know the chemical integrity of the annatto seed color. The FTIR study of annatto seed color with a mixture of tablet excipients and color with the model drug diclofenac sodium were carried out to find out the physical and chemical interaction between the annatto seed color with the excipients and the drug (Mukherjee, et al, 2006). The toxicity study of the color extract was done in Albino Rat by oral administration of 10% w/v solution of seed extract and observed for one month. No death or abnormal behavior was noticed.

### Materials and Methods:

The chemicals used for the study such as potassium hydroxide, methyl orange and sucrose purified, isopropanol, methylene chloride, polyethylene glycol, propylene glycol and hydroxy propyl methyl cellulose were purchased from E. Merck (India) Ltd., Mumbai, India. The dried ripen Annatto seeds (*Bixa orellana*, Family- *Bixacea*, Fig.-10) were collected in the month of June 2005, from the forest area of Koraput District, Orissa, India. The collected seeds were identified with the local weaver / dyers and by consulting related books on dye yielding plants.

### Extraction of Color:

To extract the color, following procedure adopted on trial basis. First of all the annatto seeds were extracted with 5% alkali solution (5%w/v of potassium hydroxide solution), which are precipitated by adding dilute hydrochloric acid and filtered to get wet mass. The wet mass was dried at 50°C temperature in a hot air oven to obtain dry powder. Weight of dry powder obtained from 50 gm of seed was found to be 4 gm, i.e. 8% color in dry weight basis. The alkali extract of annatto seed is water soluble, but the acid precipitated dried annatto seed powder is found to be insoluble in water and soluble in organic solvents like ethanol, methanol, isopropanol, methylene chloride, etc.

### Characterization and Standardization of Color:

#### Determination of wavelength ( $\lambda_{max}$ ) of Annatto Seed Extract

A double beam UV-Visible spectrophotometer (Elico-SL-164) with matched quartz cell of 1 cm path length was used to determine the  $\lambda_{max}$ . A 10ppm

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solution of annatto seed extract was prepared in 5%w/v of potassium hydrochloride solution and scanned in U.V.-Vis range, where 5%w/v of potassium hydrochloride has been taken as reference. From the spectral analysis, the maximum absorption found to be occurring at the wavelength of 453nm (Fig-1) was selected for further work.

#### Determination of wavelength ( $\lambda_{max}$ ) of methyl orange

A 10ppm solution of methyl orange was prepared and scanned by double beam UV-Vis spectrophotometer (Elico- SL-164) with matched quartz cell of 1 cm path length in UV-Vis range, where water has been taken as reference. From the spectral analysis, the maximum wavelength found to be 465nm, Fig-2, which is nearer to that of the annatto seed extract.

#### Preparation of standard curve of Annatto seed Extract

Different concentrations of solutions of alkaline annatto seed extract such as 10ppm, 20ppm, 30ppm, 40ppm and 50ppm were prepared. The respective absorbance of the prepared solutions was measured

#### Comparative study of annatto seed extract with methyl orange

The color intensity of annatto seed extract was compared with that of the methyl orange which was used as reference standard. Different concentrations such as 10ppm, 20ppm, 30ppm, 40ppm and 50ppm solutions of annatto seed extract and methyl orange were prepared and scanned by UV-Vis spectrophotometer (Elico-SL-164) at 453nm and 465 nm, respectively. Their absorbances were compared to know the color intensity of annatto seed extract with that of the methyl orange (Fig-3 and Fig-4).

#### Stability study of annatto seed extracts in the oral liquid dosage forms: (in simple syrup)

Preparation of simple syrup:

Sucrose: 850 gm

Purified water up to:1000 ml

The sucrose was placed in a percolator and the neck of which was nearly filled with loosely packed cotton, moistened with a few drops of water. About 450 ml of purified water was poured upon the sucrose and regulated the outflow to a steady drip to percolate. The procedure was continued until all of the sucrose being dissolved. Then the inside of the percolator and the cotton were washed with sufficient purified water to bring the volume of the percolate to 1000ml.

#### Stability Study at Ambient Temperature

0.1 to 0.5% w/v concentrations of colored solutions were prepared with simple syrup and kept for 7 days at ambient temperature. No aggregation and no color fade were observed after 7 days. The absorbance were measured with the appropriate dilution with simple syrup as blank and converted to colour concentration with respect to standard curve of annatto seed extract with double beam UV-Visible spectrophotometer (ELICO-SL-164) at 453nm (Table No. 1).

#### Stability Study at Accelerated Conditions

A 100ppm solution of annatto seed extract with sugar syrup was prepared and divided into three parts 100ml each and labeled accordingly. Each of the product placed in the humidity oven (Rolex Scientific Engineering, Ambala Cant., India.), controlled to different temperatures and relative humidity conditions such as 45° C  $\pm$  2° C / 70  $\pm$  5% relative humidity, 60° C  $\pm$  2° / 75  $\pm$  5% relative humidity and 70° C  $\pm$  2° C / 80  $\pm$  5% relative humidity. The samples were withdrawn after 0, 1, 2, 3, 4, 5, 6 and 7 days time period. The absorbances of the respective solutions were measured at 453nm using double beam UV-Vis spectrophotometer by taking simple syrup as blank and converted to color concentration with respect to standard curve of annatto seed extract. The %concentration remaining to

**Table No. 1: Stability study of Annatto seed extract with simple syrup at ambient Temperature**

No of Days	%ge w/v Concentration of Annatto Seed Extract in simple syrup									
	0.1		0.2		0.3		0.4		0.5	
	OD	Conc <sup>d</sup>	OD	Conc <sup>d</sup>	OD	Conc <sup>d</sup>	OD	Conc <sup>d</sup>	OD	Conc <sup>d</sup>
1	.658	0.1	1.306	0.2	1.9	0.3	2.51	0.4	3.1	0.5
2	.658	0.1	1.306	0.2	1.9	0.3	2.51	0.4	3.1	0.5
3	.658	0.1	1.306	0.2	1.9	0.3	2.51	0.4	3.1	0.5
4	.658	0.1	1.306	0.2	1.9	0.3	2.51	0.4	3.1	0.5
5	.658	0.1	1.306	0.2	1.9	0.3	2.51	0.4	3.1	0.5
6	.658	0.1	1.306	0.2	1.9	0.3	2.51	0.4	3.1	0.5
7	.658	0.1	1.306	0.2	1.9	0.3	2.51	0.4	3.1	0.5

♦ The O.D. was measured with 100 time dilution.

♦ The Values are the average of three readings.

**Table No. 2: Determination of %ge Conc. Un-decomposed vs. time,  $K^0$  &  $t_{90}$  at 45°C, 60° C & 70° C for Annatto seed extract (Accelerated Stability Study with Simple Syrup)**

Time in Days	45° C			60° C			70° C		
	%conc.	$K^0$ Values	$t_{90}^{0.1a}$ / $K^0$ in Days	% conc.	$K^0$ Values	$t_{90}^{0.1a}$ / $K^0$ in Days	% conc.	$K^0$ Values	$t_{90}^{0.1a}$ / $K^0$ in Days
1	100			100			100		
2	100			99.9			99.9		
3	100			99.9			99.8		
4	99.9	0.03333	300	99.8	0.0889	112	99.6	0.1069	93
5	99.9			99.7			99.5		
6	99.8			99.6			99.4		
7	99.8			99.5			99.4		

**Table No. 3: Chemical Composition of Various Annatto Seed Color Coating Solutions for Coating on Tablets and Microcapsules, etc.:**

Ingredients	Formula-1 (for 100 ml) 'F1'	Formula-2 (for 100 ml) 'F2'	Formula-3 (for 100 ml) 'F3'
Hydroxy Propyl Methyl Cellulose	4.0 gm.	4.0 gm.	4.0 gm.
Isopropyl alcohol	45 ml.	45 ml.	45 ml.
Methylene Chloride	55 ml.	55 ml.	55 ml.
P. E. G-4000	0.05 gm.	0.05 gm.	0.05 gm.
Titanium dioxide	01 gm	01 gm	01 gm
Propylene glycol	0.05 ml.	0.05 ml.	0.05 ml.
Annatto Seed Color (powdered)	0.05 gm	0.1 gm	0.15 gm

decompose vs. time plot, the  $K^0$  values and  $t_{90}$  were determined to identify the color stability at normal temperature i.e. at 25° C (Table-2).

#### Stability Study of annatto seed color in solid dosage forms (in tablets):

To study the physical and chemical color stability and integrity of annatto seed color, uncoated tablets containing diclofenac sodium calculated to dose strength of 50mg were prepared with starch and other excipients and different colored strength of film coating formulations with hydroxyl propyl methyl cellulose were prepared (table-3; fig.-9) and coated by coating pan, controlled to 16 rpm with blowing hot air (controlled to temperature at 50 °C). Various physical and chemical parameters (Vijayalaxmi, et al., 2004) as well as accelerated stability study at 40°C $\pm$ 2°C/ 75 $\pm$ 5%RH according to ICH Q1A (R) guideline and sampling points of 0, 3 and 6 months for drug products (Saha, et al, 2007) of uncoated and colored film coated tablets in its final packaging conditions in aluminum foil pack were studied (table- 7) to

Table No. 4: Physico-chemical parameters of uncoated (F) and color film coated (F1, F2, and F3) tablets:

Parameters	F	F1	F2	F3
*Thickness	3.37±0.04	3.61±0.0	3.6±0.0	3.6±0.0
*Diameter (mm)	9±0.0	9±0.0	9±0.0	9±0.0
#Weight (mg)	187±4.08	191.8±3.35	191.4±1.07	191.2±1.02
#Hardness (kg/cm <sup>2</sup> )	1.76±0.04	3.48±0.05	3.5±0.03	3.48±0.05
Friability (%)	0.3	0.0	0.0	0.0
*Disintegration Time (min)	0.5±0.0	0.76±0.02	0.77±0.02	0.78±0.02
Weight gain(mg) at RH : 75±5%	10	1.2	1.3	1.2
% Assay (%)	99.13±0.13	99.29±0.84	98.82±0.47	99.52±0.02
%age <sup>1</sup> Drug content at 40°C±2°C/ 75±5%RH at 3 month	98.05±0.01	99.03±0.02	98.09±0.07	99.17±0.47
%age <sup>2</sup> Drug content at 40°C±2°C/ 75±5%RH at 6 month	96.05±0.04	98.04±0.04	97.11±0.08	98.02±0.02

F: Uncoated tablet, F1: Colored film coated tablet with 0.05% w/v of annatto seed color, F2: Colored film coated tablet with 0.1 % w/v of annatto seed color, F3: Colored film coated tablet with 0.15 % w/v of annatto seed color, RH = Relative Humidity, \*Average of 6 determinations with standard deviation, # Average of 10 determinations with standard deviation, <sup>1</sup>Average of 3 determinations with standard deviation.

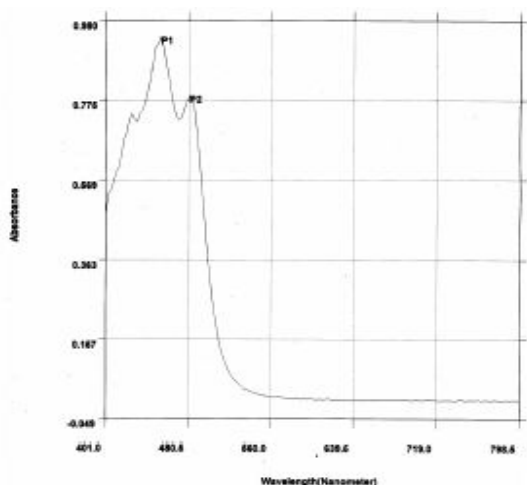


Fig. 1: Scan of Annatto seed color

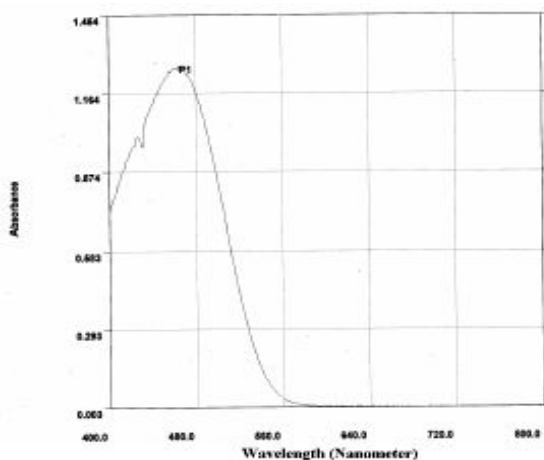


Fig. 2: Scan of Methyl Orange.

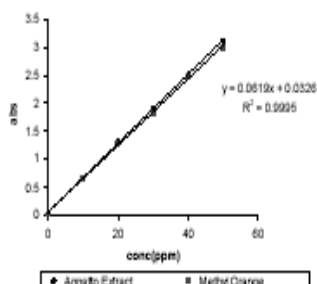


Fig. 3: Comparative color intensity study of Annatto seed color with methyl orange



(a) Annatto Color (b) Methyl Orange

Fig. 4: Comparative color intensity study of Annatto seed color (100 ppm) with methyl orange (100 ppm).

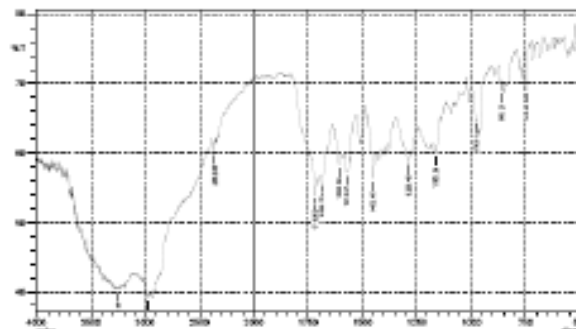


Fig. 5: FTIR spectrum of Annatto seed color.

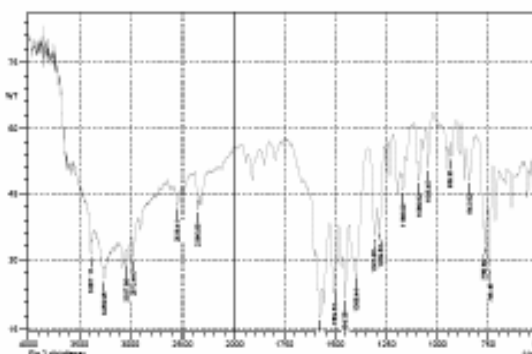


Fig. 6: FTIR spectrum of Diclofenac sodium.

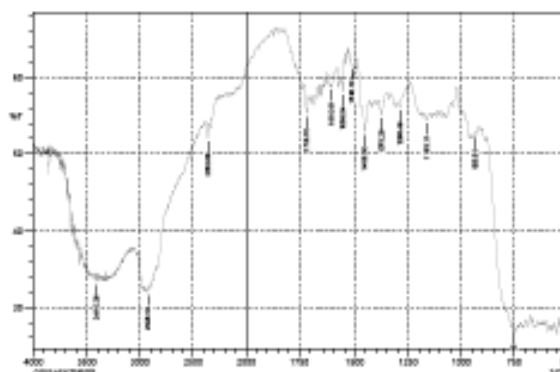


Fig. 7: FTIR spectrum of Annatto Seed Color with excipients.

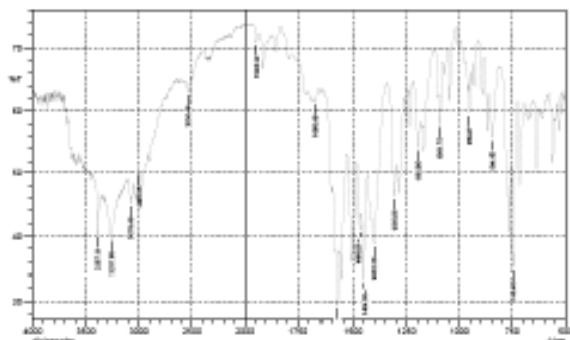
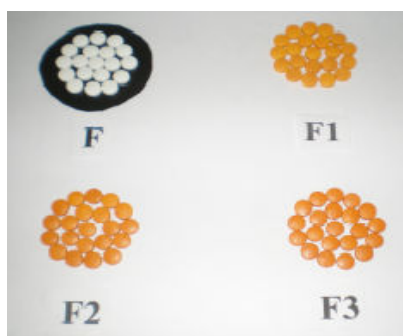


Fig. 8: FTIR spectrum of Annatto seed color with Diclofenac sodium

Fig. 9: Uncoated and colored film coated tablet with different concentration of Annatto seed color.



F1: Uncoated tablet, F2: colored film coated tablet with 0.05%w/v of Annatto seed color,  
F2: Colored film coated tablet with 0.1%w/v of Annatto seed color,  
F3: Colored film coated tablet with 0.15%w/v of Annatto seed color.



Fig. 10: Annatto Plant (*Bixa Orellana*, Fam : Bixacea)

assess the physico-chemical stability and integrity of annatto seed color in oral solid dosage forms like tablets.

#### Drug content determination:

For drug content, 20 tablets were weighed accurately and powdered. Powder equivalent to 50mg of diclofenac sodium was shaken with 60ml of methanol in 200ml volumetric flask and volume was further adjusted with methanol. Finally 5ml of this was diluted to 100 ml in volumetric flask and drug content were determined by UV-Vis spectrophotometer (ELICO-SL-164) at 276nm using calibration curve based on standard solutions(The Indian pharmacopoeia, 1996).

#### Procedure of color film coating with annatto seed color:

Required quantity of HPMC (as per formula given in table-3) dissolved in isopropyl alcohol and PEG-4000 dissolved in methylene chloride and added to the above solution. Required amount of propylene glycol and titanium dioxide was added to the solution and mixed well.

Required quantity of annatto seed color (powdered) added to the above solution with proper mixing to distribute the color. Now the above solution was used for coating by simple conventional coating pan at 16 rpm and dried by blowing hot dry air over the tablet bed controlled to 50°C temperature. The fig-8 shows the different color hues on the tablet starting from yellow to orange red color with different color strength of powdered annatto seed color.

#### Toxicity Study:

To study the toxic effect (if any) of annatto seed extract, the toxicity study was conducted on 12 male Albino rat with an average weight of 89.58gms. The animal experiments were conducted following the guideline of institutional animal ethics committee. The animals were housed in polypropylene cages at 25° C ± 2°C / 60% relative humidity in normal day and night photo cycle. The animals were fastened for 12 h before oral administration of the colored solution. The animals were given 1 ml of 1.0% solution orally and were monitored for 24 h. The animals are then had free access to basal diet (Mukherjee et al. 1998) and continued administration of 1 ml of 1.0% solution orally daily for 7 days and were observed for one month. No death or abnormal behavior was noticed.

#### FTIR Study: (Mukherjee, et al., 2006)

To study the annatto seed color with tablet coating excipient and drug interaction, the pure color, a mixture of color and excipients and color with model drug diclofenac sodium were mixed separately with IR grade KBr in the ratio 1:100. The well-ground and mixed powdered samples were compressed into pallets and the pallets were scanned over a wave number of 4000 to 500 cm<sup>-1</sup> in a FTIR instrument (Shimadzu, Japan.). The Fig.-5, 6, 7 and fig-8 shows the FTIR spectrum of annatto seed color, diclofenac sodium (model drug) and color with excipients and color with diclofenac sodium respectively. When the figures were compared, it was found that there were no major physical and chemical interaction between the annatto seed color with the excipients and the drug. However there were some minor changes in the wave number between 4000 and 2800 cm<sup>-1</sup>. Wave numbers between 4000 and 2800 cm<sup>-1</sup> are the stretching zone of -OH (hydroxyl group) (3600-3200 cm<sup>-1</sup>), C-H (alkenes) (3100-3000 cm<sup>-1</sup>), C-H (aromatic) (3000- 2500 cm<sup>-1</sup>), C=O (keto ) (1725- 1705 cm<sup>-1</sup>), C-OH(alcohol)(1150-1040cm<sup>-1</sup>) and wave number between 1690 and 1620 cm<sup>-1</sup> are the stretching zone of C=N ( 1668- 1610 cm<sup>-1</sup>). This may be because of low intensity physical bond formation between the compounds. These functional groups are present in the annatto seed color component like bixin and nor-bixin, carbohydrates (HPMC) and diclofenac

sodium. So from IR spectroscopic study, it was found that there were no evidence of interaction between the color, excipients and the drug diclofenac sodium.

### Results and Discussion

From the fig-1, the  $\lambda_{\text{max}}$  for alkaline Annatto seed extract was found to be 453 nm. From the Fig-2, it was observed that the  $\lambda_{\text{max}}$  for standard methyl orange solution was 465nm, which is nearer region to that of the annatto seed extract. From the fig-3 and fig-4, it was observed that the color intensity of annatto seed extract is more in comparison to that of the methyl orange. From the table-1, it was observed that the color of Annatto seed extract is stable at room temperature in liquid formulation. From the Table-2, it was observed that the color is stable at higher temperature and humidity conditions, which are suitable for pharmaceutical preparations. Percentage concentration remaining to decompose with respect to time (table-2) suggests that the color fading (color loss) of the liquid preparation follows zero order kinetics and the days to 90% of initial concentration Vs temperature indicate that the color is stable up to 3 months (approx) at 70°C, 4 months (approx) at 60°C and 10 months (approx) at 45°C. The extrapolation of the data indicates that the annatto seed extract is stable in liquid dosage form in syrup base up to two years at normal temperature i.e. at 25 °C, which is suitable for oral liquid dosage forms. From the physical and chemical parameters and accelerated stability data (table-4) it was observed that the color was stable at accelerated temperature and relative humidity conditions in solid dosage form such as tablets and strength of the tablets increased with colored film coating without much interfering with its disintegration time. The chemical interaction was detected between the color, excipients and the model drug diclofenac sodium from FTIR spectral analysis (fig-5, fig-6, fig-7 and fig-8). The fig-9 shows the different color hues starting from yellow to orange red color with different color strength of powdered annatto seed color. From the toxicity study, it was observed that no death or abnormal behavior of animals occurs, indicating the color material is nontoxic at the applied dose. These above facts indicate the suitability of using annatto seed color in oral solid as well as liquid dosage forms.

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### References

- Aulton, M. E., 2002. The Science of Dosage from Design, 2nd Edition, Churchill Livingstone, New York, U.S.A., p: 4.
- Bell, T., 1991. Colorants and drug reactions [letter]. *Lancet*, 338, pp: 55-56.
- Code of Federal Regulations, 2002. U. S. Govt. Printing Office, Title 21, Vol-1: 346-347.
- Dietemann Molard, A., Braun, J. J., Sohler, B., and Pauli, G., 1991. Extrinsic allergic alveolitis secondary to carmine [letter]. *Lancet*, 338: 460.
- Garrett, E.R. and Carper, R.F., 1955. *J. Am. Pharm. Assoc., Sci*, 44: 515.
- Hess, H. and Schrank, J. 1979. Coloration of pharmaceuticals: possibilities and technical problems. *Acata Pharm Technol*, 25 (Suppl.8), pp: 77-87.
- John S. Cannell, 2000. Stability Testing in Buffer, Poucher's perfumes, cosmetics and soaps, 10<sup>th</sup> Edn., Academic Publishers Kluwer, Great Britain. pp: 697-713.
- Kalsec-Natural colors, Annatto, 2002. Kalsec® publication, U.S.A., pp: 1-2.
- Leon Lachman, et al., 1987. The Theory & Practice of Industrial pharmacy, 3<sup>rd</sup> Edn., Varghese Publishing House, Mumbai, India, p: 762.

Levesque, H., Moore, N and Courtois, H., 1991. Reporting adverse drug reactions by proprietary name [letter]. *Lancet* 338:393.

Martin, A., 2006. Physical Pharmacy & Pharmaceutical Sciences, 5<sup>th</sup> Edition, Lippincott, Williams & Wilkins, New York, U.S.A., pp: 428-429 and 633..

Minnguez-Mosquera, M. I., Hornero-Mendez. D and Garrido-Fernandez, J., 1995. Detection of bixin, lycopene, canthaxanthin, and beta-apo-8'-carotenal in products derived from red pepper. *J AOAC int.* 78: 491-6.

Mukharjee, B., Samanta, A and Dinda, S. C., 2006. Gum Odina- A new Table Binder. *Trends in Applied Sci. Res.*, 1: 304 – 316.

Pollock, I., Young, E and Stoneham, M., 1989. Survey of coloring and preservatives in drugs. *Br Med J*, 299: 649-651.

Remington's 2006. The Science and Practice of pharmacy, 21<sup>st</sup> edition, vol. I, p: 1031-71.

Saha, P., Mashru, R and Rane, Y., 2007. Stability testing of pharmaceuticals– A Global perspective. *J Pharma. Res.*, 6: 1-9.

Satyanarayana, A., Rao, P. P., Balaswamy, K., Velu, V. and Rao, D. G., 2006. Application of annatto dye formulations in different fruit and vegetable products. *J Food Services*, 17: 1-5.

The Indian Pharmacopoeia, 1996. Vol-II, Govt. of India, Ministry of Health and Family Welfare; The Controller of Publication, New Delhi, India. p: 244.

Vijayalaxmi, Prasad, G. S., and Devi K., 2004. Development of Alginate Based Aqueous Film Coating Formula for Tablets. *Ind. J. Pharma. Sci.* 66: 125-129.

Winter, M. and Bernstein I. L., 1989. Adverse Reactions to Drug Formulation Agents: A Hand book of Excipients. Marcel Dekker, New York, U.S.A., pp: 159-165.