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PHOTOSTABILITY EVALUATION OF A UNANI FORMULATION *SUFOOFE SAILAN*

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ABSTRACT

Photostability study is an important component of stability study of pharmaceutical products as the loss of potency of the product may be the result of drug photodecomposition and drug product becomes therapeutically inactive. Adverse effects due to the formation of minor degradation products during storage and administration have been reported. Thus a well-designed photostability studies ensure the quality of the product throughout its shelf-life and guarantee its safety, efficacy, and acceptability to the patient. In present study photostability of a Unani formulation *sufoofe sailan* (SS) a polyherbal powder preparation was carried out. Test drug contains *gule dhawa, gule fofal, mochras, gond molsri* and *suagr* and used in Unani medicine to treat gynaecological diseases. Test drug was prepared in house and evaluated for base line charactors by physico chemical parameters, HPTLC analysis and microbiological analysis. For photo challenge test drug was packed in two air tight PET container as the drug is available in market and kept in stability chamber. One pack was exposed to overall illumination of 1.2 million lux hours and UV energy of 200 watt hours/square meter. Another pack was exposed to 2.4 million lux hours with UV energy of 400 watt hours/square meter. During the study stability chamber was run at 40±2°C and relative humidity at 75±5%. Physico chemical parameters tested do not show more than 5% change, densitometric HPTLC analysis showed minimum changes, microbiological analysis i.e. total bacterial count, total fungal count was under the limit set by WHO and specific pathogens were absent. As the physico-chemical changes were less than 5%, and microbial count was within limits mentioned by WHO guideline, SS confirmed to the ICH Guideline for photostability testing of pharmaceutical product.

KEY WORDS: Photostability study; *Sufoofe sailan;* UV light; Fluorescent light.

INTRODUCTION

Things lose their potency and efficacy with every passing second coupled with impact of varying degree of environmental conditions. Light is an important environmental factor responsible for degradation of material. Active drug molecules finished pharmaceutical products as well as its packaging material are not spare of environmental influences. US FDA and ICH guidance for industries mentioned that " the intrinsic photostability charactorstics of new drug substance and product should be evaluated to demonstrate that, as appropriate, light expouser dose not ruselts in un acceptable change in". The term "photostability" is used to describe how a compound responds to light exposure and includes not only degradation reactions but also other processes such as formation of radicals, energy transfer, and luminescence.[1] Photostability testing of the drug substance is undertaken to evaluate the overall photosensitivity of the material for development and validation purposes and to provide information necessary for handling, packaging, and labeling. A photostability assay for pharmaceutical products should provide information related to the practical use of the product, i.e., light-exposure conditions that the product will experience under its normal applications. Demand is also increasing for photoreactivity data in order to address photo-safety assessments and labelling requirements for potentially photoreactive drugs.

Due to the increasing adverse drug effects world population is turning back to traditional system of medicine and according to WHO about 80% of world population is using herbal medicines for their primary care in developing countries. Since last one decade world has seen tremendous growth in herbal drug market, majority of them comprising of polyherbal formulations but India's contribution is less than 1% to the global herbal market. Major impediment to this is lack of standardisation and deficient quality control that is to be answered to stand in the global market and globalise Indian system of medicine.

Sufoofe sailan (SS) is used to treat various gynecological disorders like sailanur *rahem* (leucorrhea), *uqr* (sterility), and *surate inzal* (premature ejaculation) in males.[27] It is a powder dosage form containing gums, flowers and sugar hence, environmental factors affects easily and thereby early degradation occurs by time. Therefore, photostability of SS was evaluated as till date no such study was carried out to confirm photodegradation.

MATERIAL AND METHODS

Procurement of raw drugs: The plant material for the test drug was procured from the herbalist/raw drug dealer at Bangalore, Karnataka, India during the month of February-July 2013, and authenticated by the pharmacognosist. A specimen of each plant material used was deposited in the drug museum, National Institute of Unani Medicine, Bangalore (voucher specimen no. 19/IS/Res./2014), for future reference. Two of the organized herbal drugs namely *gule dhawa (Woodfordia fructosa* L. Kurz.) and *gule fofal* (*Areca catechu* L.) were further certified by Dr. Sumathi, Herbarium curator, Department of Botany, FRLHT, Bangalore (accession number: 2968 and 2969 respectively).

Preparation of *sufoofe sailan*: All ingredients (listed in Table 1)were rinsed with running tap water and shade dried at 60°C in hot air oven prior to use. Each ingredient was ground separately in the electric grinder and passed through no. 80 mesh sieve. Then these powdered ingredients were weighed separately in the ratio mentioned in NFUM and mixed rigorously in electric kitchen mixer to get homogenous powder.

Table 1: Ingredients of *sufoofe sailan*[5]

S.no.	Drug name	Botanical name	Part used	Proportion
1.	*Gule dhawa*	*Woodfordia fructosa* L.Kurz.	Flower	12.5%
2.	*Gule fofal*	*Areca catechu* L.	Flower	12.5%
3.	*Mochras*	*Bombax malabaricum* Dc.	Gum	12.5%
4.	*Gond molsri*	*Mimusops elengi* L.	Gum	12.5%
5.	*Nabat safaid*	Sugar	Crystals	50%

Storage: Air tight container closure system of 250 ml capacity, made up of transparent polyethylene terephthalate (PET), procured from local market was used for storage purpose. About 200 gm of drug formulation was filled into the container, covered with aluminium foil and tightly closed with red polypropylene threaded cap**.** All precautions were taken while packaging test drug samples in the containers like containers were cleaned, dried, covered with aluminium foil and fitted with air tight lids properly.

Photostability testing: Two packs of SS were subjected to photostability testing using Osworld photostability chamber (Model- Osworld photostability chamber OPSH G-4 1258).. One pack was exposed to overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200 Watt hours/square meter. Another pack was exposed to 2.4 million lux hours with an integrated near ultraviolet energy of 400 Watt hours/square meter. That was

calcualtaed as one sample was exposed to fluorescent light for 4 days 12 hours and UV light for 22 hours 13 minutes to achieve 1.2 million lux hour fluorescent light and 200 Watt hours/square m[2] UV light. Another one sample was exposed to double time duration to this. During the photostability study stability chamber was run at 40±2°C and relative humidity at 75±5%. Calculation of exposure time for fluorescent light and UV light is given in table 2 and table 3. A cool white fluorescent lamp designed to produce an output similar to that specified in ISO 10977(1993) and near UV fluorescent lamp having a spectral distribution from 320 nm to 400 nm with a maximum energy emission between 350 nm and 370 nm and significant proportion of UV in both bands of 320 to 360 nm and 360 to 400 nm were used.[6]

Table 2: Calculation of exposure time for fluorescent light

Duration of Calibration	**1 hour**
Average light intensity observed in stability chamber	11100 lux
Required light intensity	1.2 million Lux hrs
Exposure required in hours	1.2 ×1000000 / 11100 = 108
Exposure required in days	108 / 24 = 4.5 Days
	= 4 Days 12 hours
Photostability Chamber (Model- Osworld photostability chamber OPSH G-4 1258).	

Table 3: Calculation of exposure time for UV light

Duration of Calibration	**1 hour**
Average light intensity observed in stability chamber	887µW/cm²/1000000=0.000887W/cm² 0.000887 W/cm² × 10000 = 8.87 W/m²
Required light intensity exposure	200 W/m²
Exposure required in hours	200 W/m² / 8.87 W/m² = 22.5
Exposure required in days	22.547 / 24
	= 22 hours and 13 minutes
Photostability Chamber (Model- Osworld photostability chamber OPSH G-4 1258).	

Physico-chemical evaluation: Prepared SS was evaluated for various parameters like organoleptic characters e.g. color,[6] odor[7] and taste.[8] Physical analysis was carried out by testing loss of weight on drying, total ash , acid insoluble ash, water soluble ash, pH of 1% and pH of 10% solution, extractive values[9] bulk and tapped density, Hausner's ratio, compressibility index.[10] Quantitative estimation was carried out for total alkaloid,[11] total glycosides[12] and total tannins.[11] Qualitative desitometric HPTLC analysis was carried out to develop the characteristic finger print of various samples of SS. For HPTLC analysis extraction of SS was done in water **:** dichloromethane (1:1) and used for TLC application.

Analysis was performed on 2.5×10cm silica gel 60 F_{254} plates. Sample solution was applied using Linomat 5 (Camag Switzerland) automated spray-on band applicator equipped with a 100µl Hamilton syringe and operated with the settings as follows: Band length 8mm, distance from the plate edge 12.5mm, and distance from the bottom of the plate 10mm. Development of the plate was carried out allowing 20 minutes for saturation of the twin trough chamber (Camag Switzerland) at room temperature. Solvent system used was toluene**:** ethyl acetate**:** formic acid (7**:**2.5**:**0.5) for mobile phase and migration was 8cm. After development the plate was evaluated under UV 254 nm and 366 nm, the plate was derivatised with anisaldehyde sulphuric acid and kept in oven at 110°C and evaluated under visible light using CAMAG TLC Visualiser and scanned using CAMAG TLC SCANNER-3.[15]

Microbial evaluation: SS was evaluated for total bacterial, total fungal count and presence of specific pathogens i.e. *Escherichia coli*, *Salmonella spp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.*[16,17]

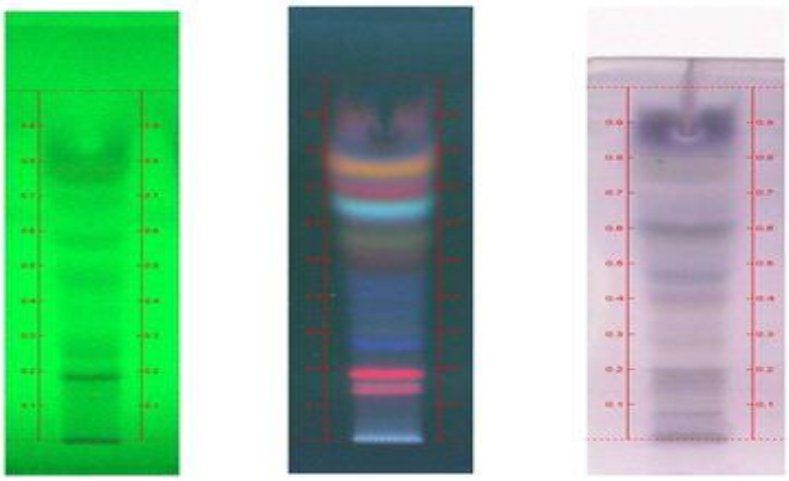
RESULTS AND DISCUSSION

Results of photostability study of SS is summarised in table 4-7. Results showed that there was no considerable variation in the formulation at forth and ninth day when compared with baseline sample. Organoleptic characters did not show any significant change. Change in bulk density, tapped density, loss of weight on drying, total ash, acid insoluble ash, water soluble ash, pH in 1% and 10%, extractive values in water, alcohol, hydroalcohol, petroleum ether and chloroform was less than 5%. As shown in HPTLC densitometry scan (figure 1-6) changes in finger prints of various sample were minimum. Total microbial count was less than the limitations offered by WHO throughout study period.

According to ICH guideline to confirm the shelf life/stability of product, change in assay from its initial value should not vary more than 5% and meet the acceptance criteria like for appearance, physical attributes etc.[18] However, even 90% of labelled potency is commonly considered as the minimum acceptable potency level.[11] In the present study, to assess the changes in physicochemical parameters of the test drug formulation, 5% variation limit was adopted. In view of the aforementioned physico-chemical and microbiological assy findings, SS confirms to the ICH Harmonised Tripartite Guideline for photostability testing of pharmaceutical product.[19] Thus it could be safely said that SS is light stable and do not undergo significant photo-degeredation.

Table 4: Organoleptic and physico-chemical characteristics of *sufoofe sailan*

Parameters	**Zero day sample 1**	**Forth day Sample 2**	**Ninth day Sample 3**
Organoleptic description			
Appearance	solid/ hard powder	solid/ hard powder	solid/ hard powder
Colour	Light brown 7.5YR5/6	Light brown 7.5YR5/6	Light brown 7.5YR5/6
Odour	Odourless	odourless	Odourless
Taste	Sweet Pleasant	Sweet Pleasant	Sweet Pleasant
Physico- chemical characteristics			
Bulk densitygm/cm^3	0.48±0.01	0.48±0.03	0.46±0.01
Tapped density	0.63±0.00	0.66±0.07	0.61±0.01
Hausner's ratio	1.29±0.01	1.37±0.00	1.31±0.01
Compressibility index	23±2.68	26.03±1.0	23.66±0.88
Total ash (%w/w)	2.57±0.02	2.52±.0.00	2.46±0..00
Acid insoluble ash (%w/w)	1.19±0.00	1.15±0.01	1.17±0.01
Water soluble ash (%w/w)	0.77±0.01	0.74±0.02	0.75±0.05
Alcohol soluble extractive value (%w/w)	22.18±0.09	22.16±0.14	21.72±0.27
Water soluble extractive value (%w/w)	22.18±0.09	22.16±0.14	21.72±0.27
Chloroform soluble extractive value (%w/w)	1.19±0.02	1.18±0.09	1.14±0.01
Petroleum ether soluble extractive value (%w/w)	0.45±0.01	0.44±0.02	0.43±0.01
Loss on drying (%w/w)	4.7±0.03	4.77±0.016	4.88±0.02
pH 1 % solution (%w/v)	4.78±0.00	4.88±0.00	4.86±0.00
pH 10% Solution (%w/v)	5.19±0.01	5.26±0.00	5.31±0.00
Total Alkaloid (%w/w)	2.58±0.00	2.57±0.01	2.52±0.01
Total Glycoside (%w/w)	0.72±0.01	0.70±0.00	0.70±0.00
Total Tannins (%w/w)	10.39±0.03	10.32±0.01	10.26±0.00



Under UV 254 | Under UV 366 | Derivatised with anisaldehyde sulphuric acid under white light

Fig 1. HPTLC finger printing of *sufoofe sailan* at baseline

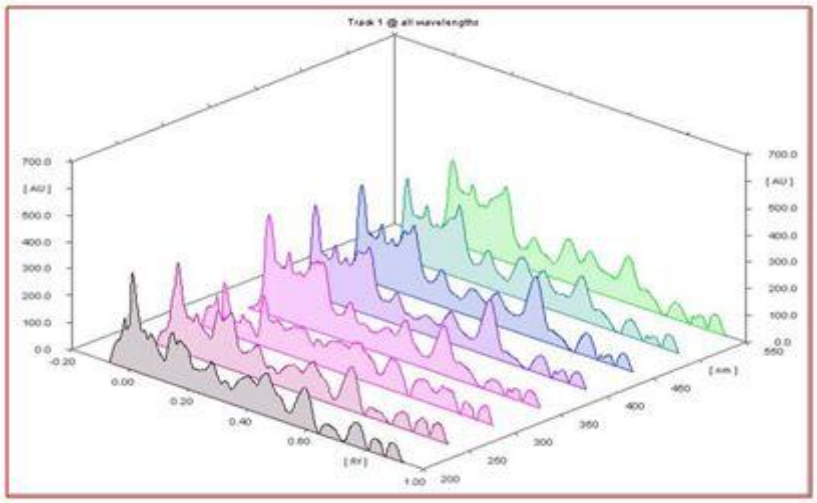


Fig 2 HPTLC densitometric scan of *sufoofe sailan* at baseline at multiple wavelengths

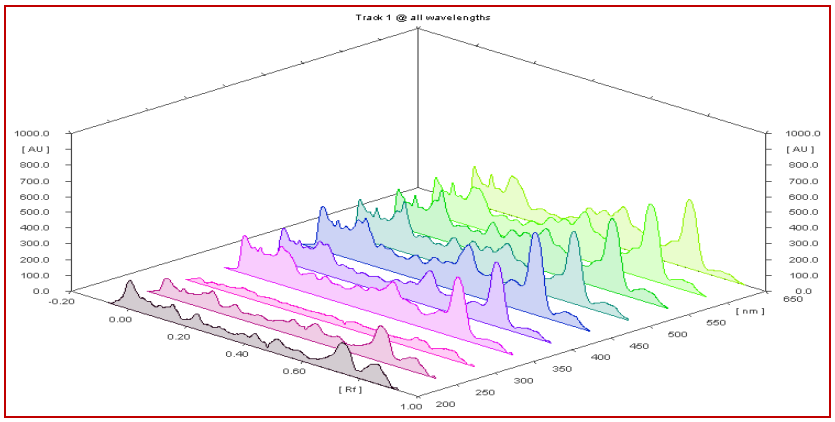
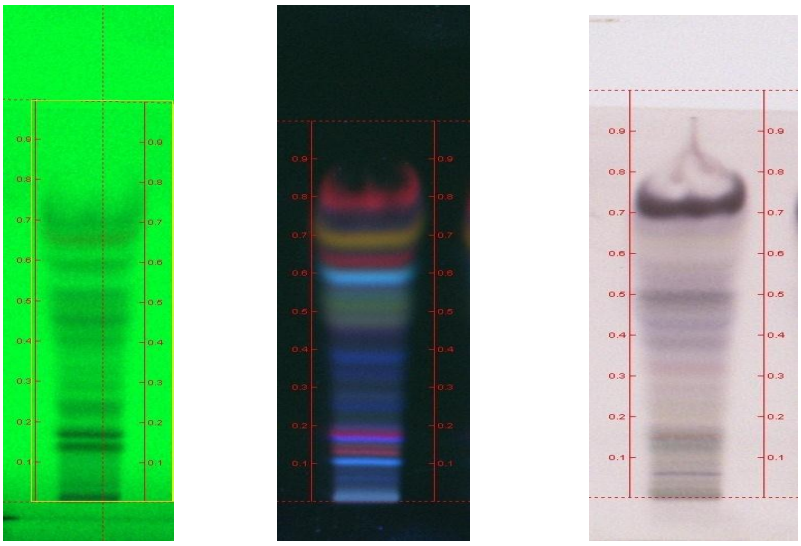
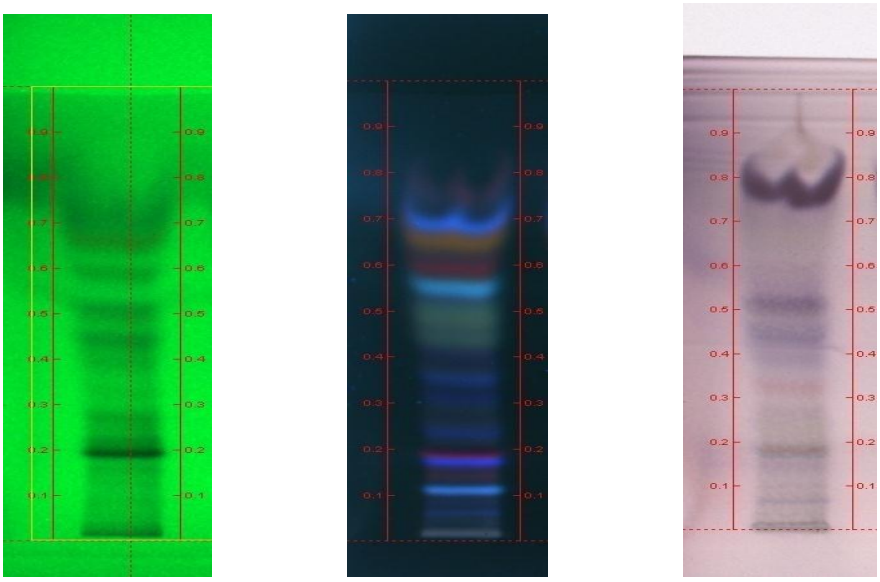


Fig 4: HPTLC densitometric scan of *sufoofe sailan* at 4th day at multiple wavelengths)



Under UV 254 | Under UV 366 | Derivatised with anisaldehyde sulphuric acid under white light

Fig 3: HPTLC profile of PSS of *sufoofe sailan* at 4th day



Under UV 254 | Under UV 366 | Derivatised with anisaldehyde sulphuric acid under white light

Fig 5: HPTLC profile of PSS of *sufoofe sailan* at 9th day

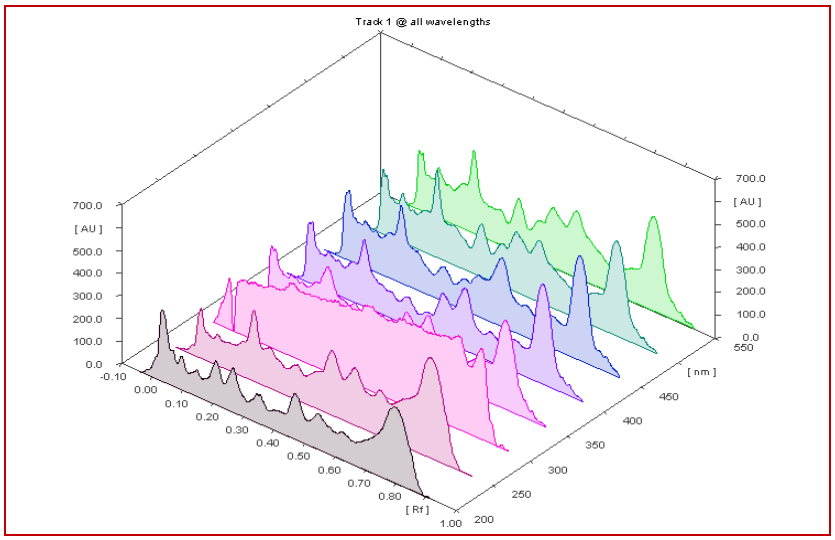


Fig 6: HPTLC densitometric scan of *sufoofe sailan* at 9th day at multiple wave length)

Table 5: *Rf* value and color of bands of PSS at zero, 4th and 9th day study under 200nm, 254nm, 366nm and after spraying anisaldehyde sulphuric acid

Day	Under UV 254nm		Under UV 366nm		After spraying anisaldehyde sulphuric acid		No. of Peaks at 200nm and their peak area and height			
	Rf value	Colour	Rf value	Colour	Rf value	Colour	No. of peaks	Rf value	Area	Height
Zero Day	0.04	Dark	0.05	Purple	0.04	Green	12	0.04	10034.43	544.60
	0.11	Light	0.09	Brown	0.07	Blue		0.09	8342.76	523.45
	0.17	Light	0.15	Blue	0.17	Blue		0.11	6221.90	487.23
	0.22	Dark	0.18	Blue	0.19	Orange		0.17	5984.38	421.35
	0.27	Dark	0.26	Brown	0.23	Yellow		0.26	4320.97	400.18
	0.32	Light	0.35	Blue	0.32	Brown		0.32	4007.31	390.32
	0.46	Light	0.40	Orange	0.36	Blue		0.40	3456.74	287.43
	0.51	Dark	0.45	Yellow	0.41	Purple		0.46	3458.53	220.10
	0.62	Light	0.55	Blue	0.48	Blue		0.55	2513.10	127.23
	0.69	Dark	0.63	Brown	0.67	Black		0.62	1854.12	104.21
			0.68	Orange				0.79	604.72	18.91
			0.89	Brown				0.89	431.56	16.23
4th Day	0.04	Dark	0.05	Purple	0.04	Green	11	0.04	10034.43	544.60
	0.11	Light	0.09	Brown	0.07	Blue		0.09	8039.76	503.15
	0.17	Light	0.15	Blue	0.17	Blue		0.11	6221.90	487.23
	0.22	Dark	0.18	Blue	0.19	Orange		0.17	5784.38	412.39
	0.27	Dark	0.26	Brown	0.23	Yellow		0.26	4320.97	400.18
	0.32	Light	0.35	Blue	0.32	Brown		0.32	4007.31	390.32
	0.46	Light	0.40	Orange	0.36	Blue		0.40	3456.74	287.43
	0.51	Dark	0.45	Yellow	0.41	Purple		0.46	3458.53	220.10
	0.62	Light	0.55	Blue	0.48	Blue		0.55	2513.10	127.23
	0.69	Dark	0.63	Brown	0.67	Black		0.62	1854.12	104.21
			0.79	Purple				0.79	604.72	18.91

9th Day	0.04	Dark	0.05	Purple	0.04	Green	11	0.04	10034.43	544.60
	0.11	Light	0.09	Brown	0.07	Blue		0.09	8039.76	503.15
	0.17	Light	0.15	Blue	0.17	Blue		0.11	6221.90	487.23
	0.22	Dark	0.18	Blue	0.19	Orange		0.17	5741.23	412.39
	0.27	Dark	0.26	Brown	0.23	Yellow		0.26	4320.97	400.18
	0.51	Dark	0.45	Yellow	0.41	Purple		0.32	3458.53	220.10
	0.62	Light	0.55	Blue	0.48	Blue		0.40	2813.70	217.13
	0.69	Dark	0.63	Brown	0.67	Black		0.46	1854.12	104.21
			0.71	Blue	0.72	Brown		0.55	1245.72	68.54
			0.79	Purple				0.62	976.89	40.23
								0.79	604.72	18.91

Table 6: Bacterial and fungal count of photostability testing samples of *sufoofe sailan*

S.No.	Sample	Total bacterial count (cfu/gm/ml)	WHO limit	Total fungal count (cfu/gm/ml)	WHO limit	Inference
1.	0^{th} day	30000	10^5	20	10^3	Within Limit
2.	4^{th} day	30000	10^5	10	10^3	Within Limit
3.	9^{th} day	70000	10^5	500	10^3	Within Limit

Table 7: Presence of pathogenic bacteria in photostability samples

S. No.	Sample	*E.coli*	*Salmonella*	*Staphyloccus aureus*	*Pseudomonas aeruginosa*
1.	Zero day	Absent	Absent	Absent	Absent
2.	Fourth day	Absent	Absent	Absent	Absent
3.	Ninth day	Absent	Absent	Absent	Absent

CONCLUSION

As the organolaptic characters and physico-chemical changes were less than 5%, and microbial count was within limits mentioned by WHO guideline, SS confirmed to the ICH Guideline for photostability testing of pharmaceutical product. However photostability results must be evaluated together with accelerated and long-term stability results. Further biologically active molecules in the formulation should be identified and its thermal/humidity and light dependent qualitative/quantitative variation along with time should be evaluated. Likewise degradation products in the samples along with its toxicity should be confirmed.

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