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Development and validation of UV spectrophotometric method of safranal

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ABSTRACT

Determination of Safranal in a fixed dosage form was carried out by UV Spectrophotometric method. The absorbance values were observed for different dilutions of drug at 308nm and which were used for the estimation of drug without mutual interference of excipients. The solvent used for the dilution was methanol. This method obeys Beer- Lambert's law in the concentration range of 2-100 µg/ml. The results of analysis have been validated statistically and the recovery studies confirmed the accuracy of this proposed method.

Key words: safranal, UV Spectrophotometry, Method validation.

INTRODUCTION

Safranal is a monoterpene aldehyde [1], formed in saffron by hydrolysis from picrocrocin during drying and storage. It is the main essential volatile oil responsible for the saffron characteristic such as odour. The average safranal content made up of 60% of the volatile fraction of the saffron [2]. Saffron is used in traditional medicine for treatment of various disorders [3,4] and safranal may at least in part be responsible for the therapeutic effects of the plant. Several different pharmacological effects for safranal have been demonstrated including: anticonvulsant[5-9], anxiolytic and hypnotic activity. Safranal also has antioxidant properties [10]. The anticancer effect of Safranal is also demonstrated [11]. The antitussive activity of Crocus sativus stigma and petal extracts and its components, safranal and crocin, has been shown. The relaxant effects of aqueous-ethanolic extracts of C. sativus and safranal and their stimulatory effect on β-adrenoceptors were also demonstrated.

HPLC methods were reported for determination of Safranal. The review of literature revealed that no method is reported for the Safranal determination by UV spectroscopy method. The present paper describes a simple, rapid, accurate and reproducible method for the estimation of Safranal in niosomal gel formulation.

MATERIALS AND METHODS

Materials: Safranal is purchased from Sigma Aldrich. methanol, other chemicals and reagents were of analytical grade. Double distilled water was used for the study.

Instruments

A Shimadzu UV-Visible Spectrophotometer (UV-1800) with a matched pair of 10 mm quartz cuvettes and Analytical Weighing Balance (BSA 224 S, Sartorius) were used for experimental purpose.

Preparation of Standard Stock Solution and Calibration Curve

Standard stock solution was prepared by dissolving Safranal in methanol to make final concentration of 100 µg/ml. Different aliquots were taken from stock solution and diluted with methanol separately to prepare series of concentration from 2 – 10 µg/ml. An independent stock solution of 5 µg/ml was also prepared. The λ max was found by UV spectrum of Safranal in methanol, in range of 200 – 400 nm and it was found to be 308 nm as shown in Fig

1. Absorbance was measured at 308 nm against methanol as blank. The calibration curve was prepared by plotting absorbance versus concentration of Safranal.

Method Development [12-14]
This method was validated with respect to linearity, range, accuracy, precision, specificity, Ruggedness.

Specificity
Identification:
The UV absorption spectrum of the sample preparation for assay is concordant with the reference spectrum of standard sample from assay preparation.

Placebo Interference:
Placebo solution was prepared in the same manner as standard and sample preparation. No interference of placebo was found.

Linearity and Range:
The prepared aliquots i.e. series of dilutions 0.001 – 0.01 µg/ml were prepared from the stock solution and were scanned for absorbance at λmax 308 nm. . Least square regression analysis was performed on the obtained data.

Accuracy*:*
Accuracy of the method is closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentration - lower concentration (LC, 80%), intermediate concentration (IC, 100%) and higher concentration (HC, 120%) were prepared from independent stock solution of 1 µg/ml and analyzed (n=10). Accuracy was assessed as the percentage relative error and mean percentage recovery. To provide an additional support to the accuracy of the developed assay method, a standard addition method was employed, which involved addition of different concentration of pure drug (2,4 ppm) to a known pre-analyzed formulation sample and the total concentration was determined using the proposed method,(n=10). The percent recovery of the added pure drug was calculated as percent recovery= [(Ct-Cs)/Ca] x 100, where Ct is the total drug concentration in the formulation sample; Ca is the drug concentration added to the sample.

Precision:
Repeatability studies were done by repeatedly observing the absorbance of standard solution containing 5 µg/ml. Inter- day and intra-day variation were studied to determine intermediate precision of the proposed analytical method.

Inter- day precision:
Inter-day precision was found out by preparing 5 µg/ml concentration of Safranal solution for three days and standard deviation was calculated.

Intra-day precision:
Intra-day precision was found out by preparing 5 µg/ml concentration of Safranal solution for six times in a day and then analyzed each time. Standard deviation was calculated.

Stability:
The sample was subjected for stability studies under room temperature. Stability studies were conducted to check whether any changes occur in absorbance with the time.

Robustness:
Robustness was determined by analyzing Safranal concentration on different days by different analysts.

RESULTS AND DISCUSSION

As shown in the fig 1. λ max of Safranal was found to be 308 nm.

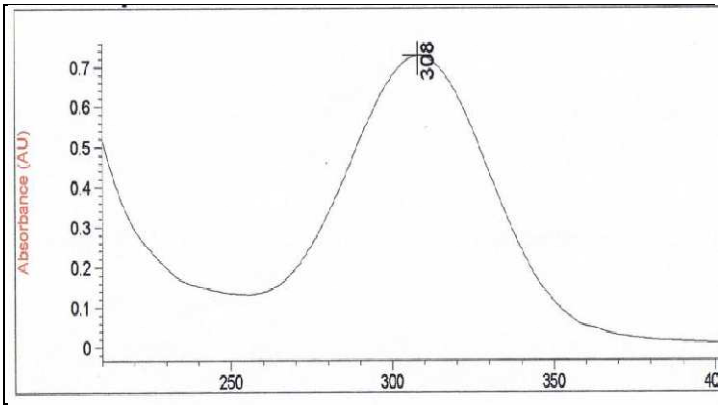


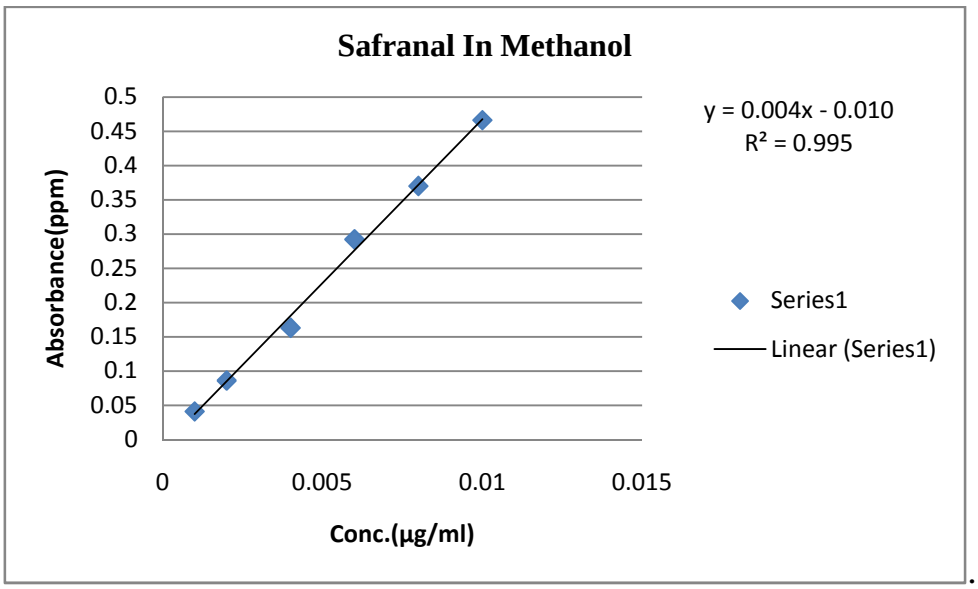
Fig. 1: UV Spectra of Safranal

Linearity and Range:

Table 1: Linearity

Sr. No.	Concentration (µg/ml)	Absorbance
1	0.001	0.041
2	0.002	0.086
3	0.004	0.163
4	0.006	0.292
5	0.008	0.370
6	0.01	0.466

Fig.2: Beer-Lambert's plot (Absorbance v/s concentration) Calibration curve



From the fig 2. It is clear that the Beer-Lambert's law was obeyed in the concentration range of 0.001-0.01µg/ml with regression coefficient (r^2value) 0.995 with the absorbance ranging from 0.041– 0.466.

Accuracy:

Table 2: Accuracy data for the developed method

Sr.No.	Level (µg/ml)	Estimated concentration (µg/ml)			Mean % Recovery (± S.D)	Accuracy*(%)
		Range	Mean(±S.D)	% RSD		
1	LC-0.002	0.00191-0.00199	0.00196±0.0015	1.28	99.1	-0.9
2	IC-0.004	0.0041-0.0042	0.0041 ±0.0028	1.39	101.66	1.66
3	HC-0.006	0.006-0.0061	0.0061±0.0025	0.95	101.11	1.11

Table 3: Standard addition of safranal for accuracy (n=10)

Sr.No.	Drug in formulation	Pure drug added	Total drug found	% Recovery
	µg/ml	µg/ml	µg/ml ±S.D	±R.S.D.
1	0.004	0	0.00396 ± 0.00014	99±0.0017
2	0.004	0.001	0.00498 ± 0.0005	99.8±0.099
3	0.004	0.002	0.00594 ± 0.002	99±0.44

The excellent mean% recovery values, close to 100% and their low standard deviation values (S.D<1.0) indicate high accuracy of the analytical methods. The validity and reliability of the proposed methods was assessed by the recovery studies. In methanol, mean % recoveries for lower, intermediate and higher concentrations were found to be 100.75, 99.4, and 99.33 respectively. The validity and reliability of the proposed methods was further assessed via recovery studies by the standard addition method (Table 3). The mean% recoveries (%R.S.D) for the intermediate concentration were found to be 99.2 (0.87), 99.6(0.74), 99.11(0.98), respectively. These reveal that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical method.

Precision:

Table 4: Repeatability data

SAFRANAL			
Sr. No.	**Concentration (µg/ml)**	**Absorbance**	**% R.S.D.**
1	0.004	0.163	3.050692
2		0.158	
3		0.173	
4		0.158	
5		0.160	
6		0.161	
7		0.160	
8		0.165	

Table 5: Inter-day Precision

SAFRANAL [Concentration (0.0004µg/ml)]		
Day	**Absorbance**	**% R.S.D.**
1st	0.159	1.290289
	0.162	
	0.163	
2nd	0.165	2.356394
	0.159	
	0.158	
3rd	0.161	1.082532
	0.161	
	0.158	

The precision was determined by studying the repeatability and intermediate precision. The repeatability results indicated the precision under the same operating conditions over a short interval of time. Intermediate precision was expressed within different days i.e. both intra- day and inter-day precision was observed. R.S.D. values for the proposed analytical method were well within the acceptable range, indicating that the method has excellent repeatability and intermediate precision

Table 6: Intra – day Precision

SAFRANAL			
Sr. No.	**Concentration (µg/ml)**	**Absorbance**	**% R.S.D.**
1	0.004	0.161	2.062951
2		0.157	
3		0.163	
4		0.167	
5		0.161	
6		0.160	

Stability:

The sample was subjected for stability studies under room temperature. The solution was stable for up to 5 hours with % R.S.D. less than 1 as shown in Table 7.

Table 7: Stability data

SAFRANAL				
Sr. No.	**Time(hr)**	**Concentration (µg/ml)**	**Absorbance**	**% R.S.D.**
1	1	0.004	0.161	1.799677
2	2		0.162	
3	3		0.158	
4	4		0.166	
5	5		0.163	

Robustness:

Table 8: Data for Robustness test

Sr. No.	Variable parameter	Assay result(%)
1	analyst 1	99.1
	analyst 2	99.3
2	day 1	98.9
	day 2	99.1

Estimation of formulation:

The estimated drug content with low values of standard deviation established the precision of the proposed method.

Table 9: Estimation of formulation

Sr . No.	Brand Name	Amount Of Drug (Mg)		% Recovery (±SD)	(%) Accuracy
		Labeled	Estimated (±SD)		
1	Safranal Niosomal Gel	25	24.48(± 0.042)	97.92(±0.16)	-2.08

** accuracy given in % relative error= [(estimated concentration- total concentration)/ total concentration] 100*

CONCLUSION

A UV-Spectrophotometric method was developed for Safranal determination. The analytical method is simple sensitive, rapid and specific and it can be conveniently employed for the routine analysis and the quality control of Safranal in pharmaceutical dosage forms. The method was suitable to determine concentrations precisely and accurately. The sample recovery from the formulation was in good agreement with its respective label claim.

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