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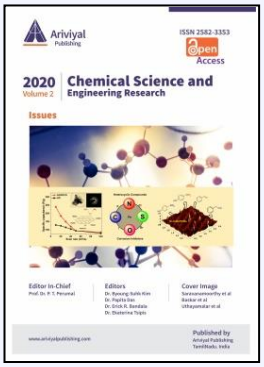

Micelle Mediated – Cloud Point Extraction and Colorimetric Estimation of Sunset Yellow in Pharmaceutical Dosage Forms

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Abstract: Sunset Yellow (SY) is a widely used pharmaceutical excipient for coloring the dosage forms. SY was efficiently extracted from selected liquid dosage forms using Brij 98 as a non-ionic surfactant in Micelle Mediated-Cloud Point Extraction (MM-CPE) method. Simple, accurate, and precise colorimetric methods like the calibration curve method and single point standardization method were developed and validated for the quantification of SY in liquid dosage forms. MM-CPE method was found to be highly efficient with good recovery rates for SY in samples and optimized for parameters like pH, the effect of salt, surfactant concentration, incubation time, and temperature. The colorimetric method showed good linearity from 3-18 µg/ml (R2=0.9995) for SY at absorption maxima of 482nm. The method was found to be precise (%RSD <2) and accurate (±10% limit). The assay values for the samples Ascoril (5ml) and P-125 drops (1 ml) were found to be 0.05 and 0.671 mg for the calibration curve method and 0.051 & 0.675 mg for the single point standardization method respectively. The developed methods were validated as per International council for Harmonisation (ICH) guidelines. The content of SY obtained is then compared with the allowable daily intake (ADI) value referred by European Union (EU) and World Health Organisation/Food and Agriculture Organisation (WHO/FAO) [0-4 mg/kg/b.w.]. The level of SY in sample dosage forms was par below the ADI limits and considered to be safe.

Keywords: Sunset Yellow; Liquid Dosage forms; Brij 98; MM-CPE; Colorimetric method

1. Introduction

Pharmaceutical products contain varied additives to modify the visual appearance, taste, or shape of the product. The appearance of these products is often modified by the addition of different colorants, thereby becoming more attractive for consumers.[1,2] Natural or synthetic food dyes are one of the most widely used groups of excipients in the pharmaceutical industry. Studies have proven that consumers form an emotional link with color.[3]

The use of synthetic and natural dyes in pharmaceuticals is strictly controlled by legislation and harmonized across the world.[4] There is a legal requirement for governments to monitor the consumption of all additives to ensure that acceptable daily intake values recommended by the World Health Organisation (ADI/WHO) are not exceeded, especially by young children.[5-6]

Certain synthetic dyes, for example, sunset yellow (SY); contain azo group and aromatic rings in their chemical structure (Fig. 1). When consumed in large amounts, these dyes pose a potential risk to human health. Some of their untoward effects include allergies, asthma, and attention-deficit hyperactivity disorder.[7-12]

Different extraction methods such as solid-phase, liquid-liquid, ultrasound-assisted and cloud-point extractions have been used to isolate SY from pharmaceutical preparations. Several analytical methods such as high-performance liquid chromatography, electrochemical sensor, spectrophotometric, liquid chromatography-tandem mass spectrometry, capillary electrophoresis, thin-layer chromatography, and immunological assay have been reported for the determination of SY.[13-19]

Non-ionic surfactants such as Triton X-100,[20] Triton X-114,[21] Brij 56,[22] and Brij 58[23] have been used for the determination of SY. To the best of our knowledge, there is no method of extracting SY with the CPE method using Brij 98 (Fig. 2), another non-ionic surfactant, in the literature. Therefore, the present work was aimed to develop a new method of CPE using Brij 98 (Polyoxyethylene -20- oleyl ether) for the first time to extract SY from liquid dosage forms and estimate using colorimetry.

2. Experimental Section

2.1. Instruments and apparatus

Absorption spectra of SY were recorded by using Shimadzu UV-1800, Japan with Computer software: UV Probe 2.33. Quartz cell of path length 1 cm was used for estimation. Calibrated analytical balance Shimadzu AY220, Digital pH meter Digisun and Ultra sonicator PCI Analytics were used in this study.

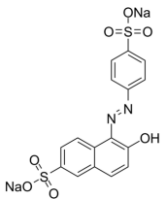


Fig. 1. Chemical structure of SY.[24-25]

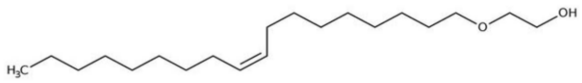


Fig. 2. Chemical structure of Brij 98.[26]

2.2. Chemicals and materials

Liquid dosage forms Ascoril (Glen mark Pharmaceuticals Ltd) and P-125 Drops (Apex Laboratories private Ltd) were purchased from the local drug store. SY (Merck Pvt Ltd, Mumbai), Brij 98 (Sigma Aldrich Pvt Ltd), etc chemicals, and calibrated glassware (class A-Borosil) were provided by Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati, Andhra Pradesh.

2.3. Extraction of SY from pharmaceutical dosage forms

This extraction is applied to test samples (dosage forms): To the test solution A, B, C; 1 ml of 20% (W/V) Brij 98, 2gms of sodium sulfate were added and final volume is made up to 10 ml with water. Stir the mixture immediately to dissolve. The mixture is then heated at 70-80^{0}C for 10 minutes until a cloudy phenomenon or separation of color is observed.

Transfer it into a 15 ml screw-capped centrifuge tube and cool down to room temperature. The mixture is now centrifuged at 4000rpm for 5 minutes for phase separation. Collect the surfactant rich top layer and discard the bottom aqueous layer using a syringe. The surfactant rich layer (residue) is diluted using distilled water for recording absorbance using a UV-Visible spectrophotometer.

2.3.1. Preparation of test solutions-

i. Test solution A (Flunarin tablet): Take one tablet, weigh and powder using a mortar and pestle and add phosphate buffer system (PBS), pH 6.8 to make up the volume to 8 ml.
ii. Test solution B (Ascoril syrup) – Take 5 ml of syrup and add PBS, pH 6.8 to make a final volume of 8ml.
iii. Test solution C (P-125 drops) – Take 1 ml of suspension add PBS, pH 6.8 and make up the volume to 8ml.

Note: Test sample is taken based on the dose of the dosage form (Fig. 3).

2.4. Optimization of extraction parameters

The extraction of SY from pharmaceutical dosage forms depends on many factors as shown (Fig. 4).

2.4.1. Effect of pH

pH is one of the vital factors corresponding to the distribution efficiency of SY. The effect of pH on SY extraction efficiency has been examined in acidic, neutral, and basic media.

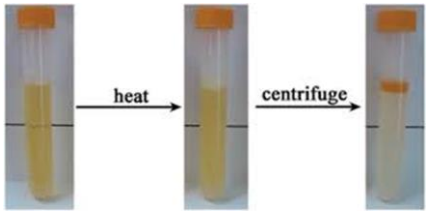


Fig. 3. MM-CPE of SY.

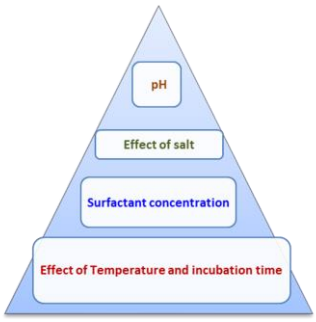


Fig. 4. Factors affecting the extraction.

2.4.2. Effect of surfactant concentration

Optimizing the concentration of the surfactant is one of the important parameters to increase the efficiency of extraction. Thus, the effect of various concentration of Brij 98 on the absorbance of SY has been studied at different concentrations as 1%, 5%, 10%, 20%, 30% w/v.

2.4.3. Type of salt

The type of salt is an important parameter in the CPE procedure because it helps the phase separation, to increase the mass transfer of the analyte from the aqueous phase to the surfactant-rich phase, and to reduce the cloud point temperature. For these reasons, the commonly used salts such as sodium chloride (NaCl), sodium carbonate (Na_2CO_3) and sodium sulphate (Na_2SO_4) have been tried and their effects on the extraction process have been investigated in our present work.

2.4.4. Effect of temperature and incubation time

Two other important parameters optimised in this extraction are the equilibrium temperature and incubation time. The temperature was examined between 50-90 °C. The incubation time was studied in an interval of 10-40 minutes.

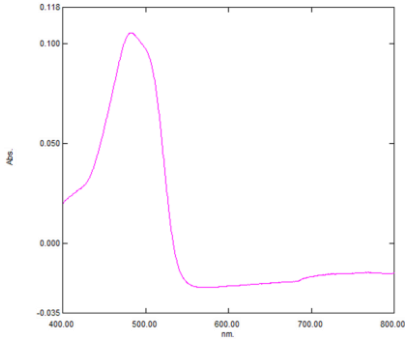


Fig. 5. Maximum absorption wavelength of SY- 482 nm

Table 1. Effect of pH on the extraction

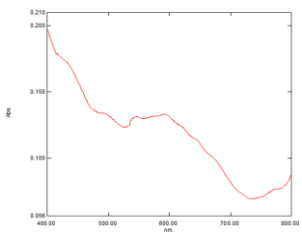
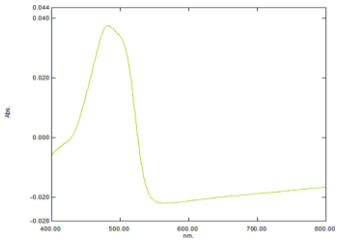
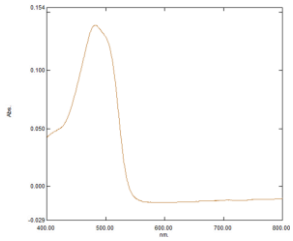
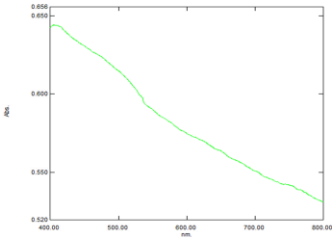
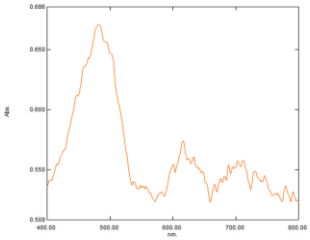
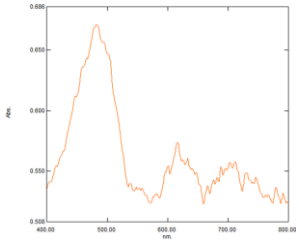
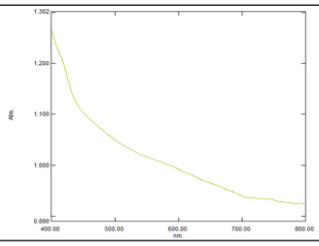
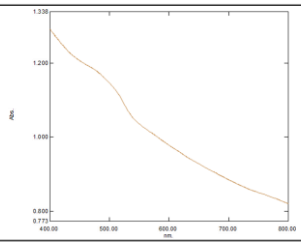
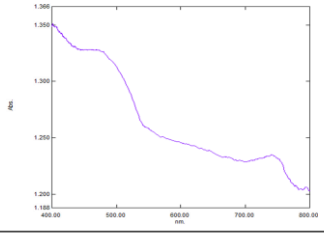
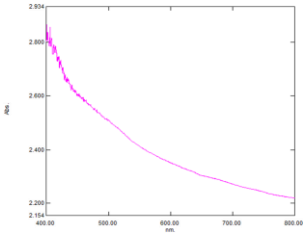
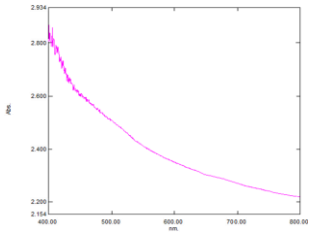
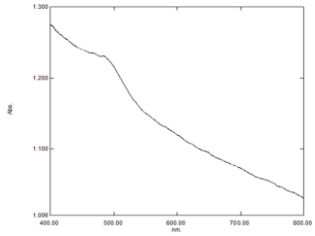
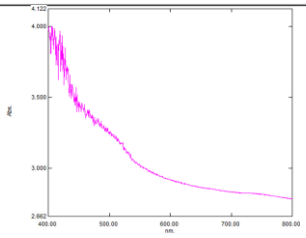
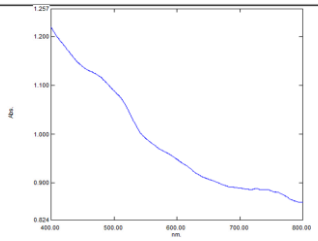
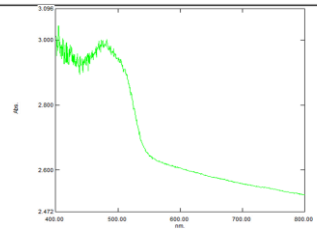
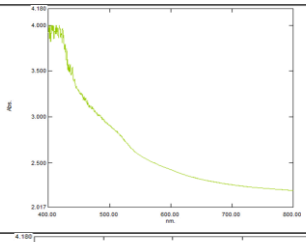
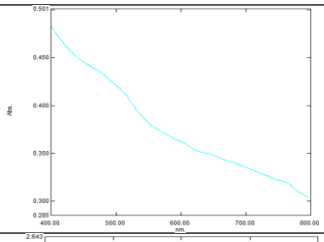
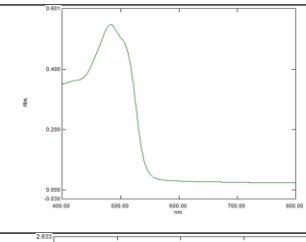
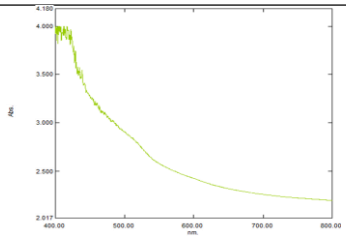
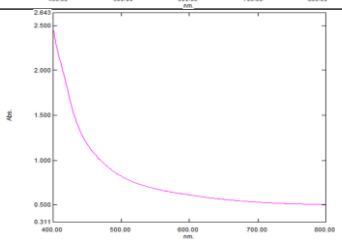
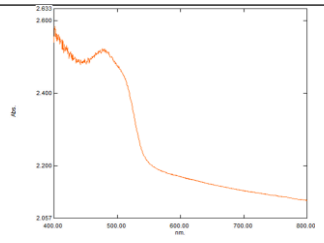
Sample	Acidic pH	Neutral pH	Basic pH
Ascoril			
P-125 drops			

Table 2. Effect of surfactant concentration and salt used

Surfactant Concentration	Sodium Carbonate	Sodium Chloride	Sodium Sulfate
1% Brij 98			
5% Brij 98			
10% Brij 98			
20% Brij 98			
30% Brij 98			

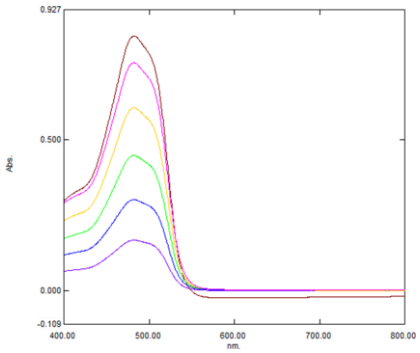


Fig. 6. Overlay spectra of standard SY at 482nm

2.5. Selection of solvent

Different solvents like water, glycerol, 0.01M ammonium acetate, and 0.1N sodium hydroxide were employed for the solubility studies. Water gave a single distinct peak at 482nm and hence water is selected as solvent.

2.6. Preparation of standard solution

To prepare 1000µg/ml standard solution, 10mg of standard SY is dissolved in 10 ml of distilled water. From this, 1ml was transferred to a 10 ml volumetric flask and made up with distilled water to obtain a stock of 100 µg/ml stock solution. The working solutions are prepared daily by diluting desired concentrations (3 to 18µg/ml) from the stock solution.

2.7. Selection of λ_{max}

From the trial and error method, the λ_{max} of standard SY was determined by preparing the solution in water and the spectra was recorded in a UV-Visible spectrophotometer. Fig. 6 shows the overlay spectra of standard SY at 482 nm.

2.8. Methods

2.8.1. Calibration Graph Method (CGM)

In the calibration graph method, the absorbances of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution.[24]

2.8.2. Single Point Standardization method (SPSM)

The single point standardization procedure involves the measurement of the absorbance of a sample solution and a standard solution of the reference substance. The concentration of the substances in the sample is calculated from the proportional relationship that exists between absorbance and concentration.[25]

$$C_{test} = (A_{test} \times C_{std})/A_{std}$$

Where,

C_{test}= concentration in the sample

C_{std} = concentration in the standard solutions

A_{test}= absorbance of the sample

A_{std}= absorbance of the standard solution

2.9. Validation parameters

The above methods are validated as per United States- Food and Drug Administration (US-FDA) and ICH guidelines.[26]

2.9.1. Linearity

The calibration curve constructed was evaluated by using the correlation coefficient. The absorbance was linear over the range of 3-18µg/ml for SY at 482 nm.

2.9.2. Accuracy

For accuracy, samples of liquid dosage form were spiked with 50%, 100%, and 150% level solutions of the standard and analyzed. The experiment was performed in triplicate. The accuracy was expressed as percentage recovery, which is determined by the standard addition method.

2.9.2.1. Procedure for preparation of test samples for percentage recovery studies:-

As there is no label claim for colorant, 1 ml of each dosage form is taken in % recovery studies. This is followed by determination of dosage form concentration and spiked concentration.

2.9.3. Precision

Variation of results within the same day (intra-day), the variation of results between days (inter-day) was analyzed. Intra-day precision was determined by analysing SY three times on the same day at 482nm. Inter-day precision was determined by analyzing at three different days at 482nm and %RSD was calculated.

3. Results and Discussions

3.1. Effect of pH

pH is an important parameter that affects the coefficient of dispersion of the analyte between the aqueous phase and the surfactant-rich phase. Upon examination of results of SY extraction efficiency in three different media acidic, basic, and neutral (Table 1) it was found that basic and neutral media are quite suitable for the separation of SY from the sample matrix, Neutral pH (6-8) is considered for our study.

3.2. Effect of surfactant concentration and type of salt

Multiple factors get involved in the optimization of colorant isolation from its sample matrix; surfactant concentration and type of salt are among them. Results obtained (Table 2) signifies that non-ionic surfactant Brij 98 at concentration of <10% w/v with sodium chloride and sodium carbonate couldn't result in the cloud formation and separation of SY. But, sodium sulfate salted out the SY surfactant cloud at all investigated surfactant concentrations, particularly at 20 % where interference-free spectra were obtained.

Table 3. Effect of temperature and incubation time on extraction

Time of incubation (minutes)	Temperature (^{0}C)			
	50-60	60-70	**70-80**	80-90
10	No separation	Cloudiness of solution	**Complete separation was observed**	Immediate separation and dissociation
20	No separation	Cloudiness and partial separation	Separated layers become sticky	Poor extraction and poor peaks formed.
30	No separation	No change in separation	No change observed	-
40	Partial cloudiness appeared	Complete separation	No change observed	-

Table 4. Recovery studies for Ascoril

Concentration spiked	Standard added	Test added	Absorbance		Concentration [T+S] (µg/ml)		Test Concentration (µg/ml)		% Recovery	
			CGM	SPSM	CGM	SPSM	CGM	SPSM	CGM	SPSM
50%	1.5 µg	1 ml of syrup	0.191	0.191	3.624	3.845	2.124	2.114	104.6%	93.7%
			0.190	0.191						
			0.191	0.190						
100%	3 µg		0.275	0.275	5.085	5.536	1.995	2.236	98.27%	99.15%
			0.255	0.273						
			0.255	0.274						
150%	4.5 µg		0.346	0.346	6.79	6.966	2.09	2.076	103.07%	92.0%
			0.345	0.346						
			0.345	0.345						

Table 5. Recovery studies for P-125 drops

Concentration spiked	Standard added	Test added	Absorbance		Concentration [T+S] (µg/ml)		Test Concentration (µg/ml)		% Recovery	
			CGM	SPSM	CGM	SPSM	CGM	SPSM	CGM	SPSM
50%	1.5 µg	1 ml of syrup	0.658	0.658	13.193	13.268	11.693	11.537	97.6%	95.50%
			0.660	0.660						
			0.659	0.659						
100%	3 µg		0.742	0.742	14.887	14.939	11.797	11.639	98.47%	96.34%
			0.741	0.741						
			0.742	0.742						
150%	4.5 µg		0.842	0.842	16.78	16.811	12.08	11.921	100.88%	98.68%
			0.821	0.821						
			0.842	0.842						

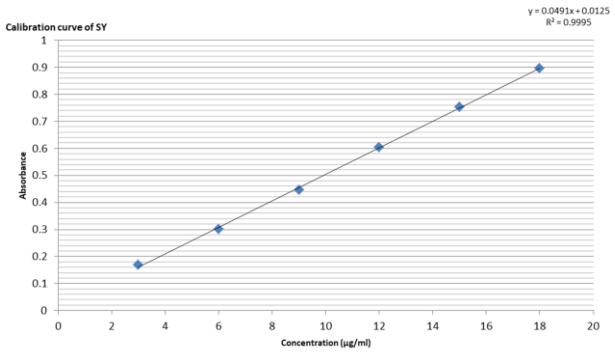


Fig. 7. Linearity profile of SY

3.3. Effect of Temperature and incubation time

Cloud point, a point of temperature at which phase separation begins, is highly influenced by change in temperature. To achieve this cloud point, temperatures from 50-900^{o}c with an incubation time at an interval of 10 min were analyzed. By studies (Table 3) it was evident that at 70-800^{o}c the solution attained cloud point temperature with good SY extraction at an incubation time of 10 min.

3.4. Validation parameters

3.4.1. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The proposed method was found to be linear (Fig. 7) in the range of 3-18 µg/ml with a correlation coefficient value (R2) of 0.9995 which states that the method was linear to the concentration.

3.4.2. Accuracy

The accuracy of method was determined by calculating mean percentage recovery at 50, 100 and 150 % level (Table 4; 5). The method was found to be accurate as percentage recovery was within limits (±10% limit).

3.4.3. Precision

In proposed method precision was studied as repeatability (%RSD<2). % RSD for Intra-day analysis of ascoril and P-125 samples was found to be 0.179% and 1.87%. %RSD for inter-day analysis of

Table 6. Intra-day precision of SY in liquid dosage forms

S.No.	Sample	Trail-1	Trail-2	Trail-3	Mean	Standard deviation	% RSD (%RSD=SD/mean)
1	Ascoril	0.556	0.557	0.555	0.556	0.001	**0.179%**
2	P-125 drops	0.671	0.681	0.656	0.669	0.0125	**1.87%**

Table 7. Inter-day precision of SY in liquid dosage forms

Sample	P-125 drops			Ascoril		
Days	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
Trail-I	0.671	0.656	0.671	0.556	0.556	0.556
Trail-II	0.681	0.635	0.681	0.557	0.556	0.554
Trail-III	0.656	0.656	0.671	0.556	0.557	0.553
Mean	0.669	0.649	0.674	0.556	0.556	0.554
SD	0.012	0.0124	0.0057	0.001	0.0005	0.0015
%RSD	1.87%	1.86%	0.87%	0.179%	0.103%	0.27%
Average %RSD		**1.53%**			**0.184%**	

Table 8. Assay Values for SY in liquid dosage forms

Sample (Based on dose)	**Ascoril sample (5 ml)**		**P-125drops (1 ml)**	
Assay values	Calibration graph method	Single point standardization method	Calibration graph method	Single point standardization method
	0.05mg	0.051mg	0.671mg	0.675mg

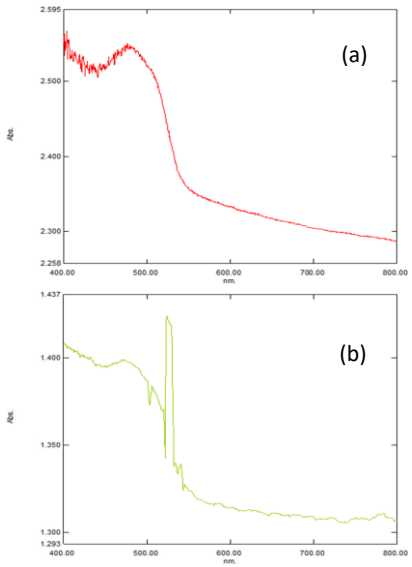


Fig. 8. Spectra of solid Sample containing SY (a) Spectra of one Flunarin Tablet after MM-CPE, (b) Spectra of one Flunarin tablet after MM-CPE following dilution

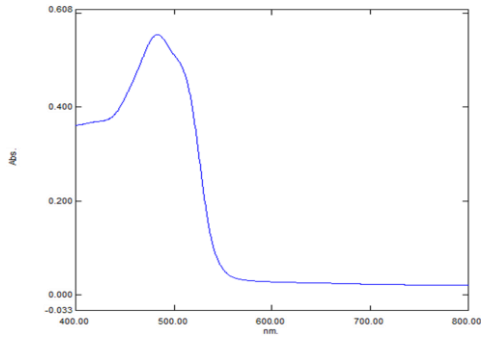


Fig. 9. Spectra of Ascoril .

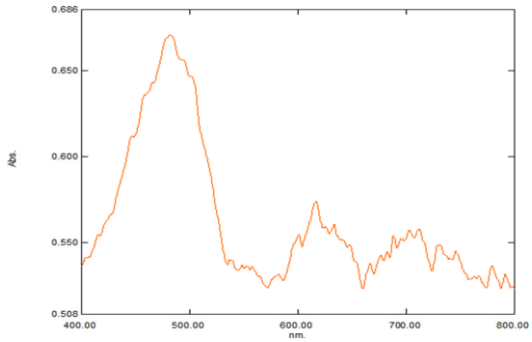


Fig. 10. Spectra of P-125 drops.

ascoril and P-125 samples were found to be 0.184% and 1.53% respectively. It is evident from the data (Table 6, 7), that % R.S.D. was found to be < 2 %; shows the high precision of the method.

3.4.4. Assay

The assay values (Table 8) for the samples ascoril (5ml) and P-125 drops (1ml) were found to be 0.05 and 0.671 mg for calibration curve method and 0.051 & 0.675 mg for single point standardization method respectively. The developed methods were simple, sensitive, accurate and precise and validated as per ICH guidelines. Hence, the developed method can be successfully applied in the extraction and estimation of SY in pharmaceutical liquid samples for routine analysis.

The content of SY obtained is then compared with allowable daily intake (ADI) value referred by EU and WHO/FAO [0-4 mg/kg/b.w.]. The level of SY in sample dosage forms are par below the ADI limits and are considered to be safe.

3.5. Absorption spectra for solid dosage form (Tablet)

In Spectra "a" - λ_{max} was seen at 477nm which deviated from λ_{max} of SY. Spectra has more interferences and absorbance was observed to be > 2.0(Beer limit : <1).

In Spectra "b" – On further dilution, the spectra did not show a distinct peak at 482nm. Therefore, colorant concentration was not determined in this dosage form.

3.6. Absorption spectra of liquid dosage form

MM-CPE method was found to be highly efficient with good extraction (Figs. 9 and 10) and recovery rates for SY in liquid dosage forms and optimized for various parameters like pH, the concentration of surfactant, type of salt, incubation time, and temperature. Spectra of the solid dosage form reveals that the MM-CPE method using 20% Brij 98 could not extract SY efficiently in solid dosage form (Fig. 8). This is probably due to the compactness of the solid matrix and other ingredients present in the solid sample. Changing the concentration of surfactant or the surfactant itself may improve the extraction of SY from the solid sample employed in this study.

4. Conclusions

SY was successfully extracted from liquid dosage forms and estimated by the colorimetric method. The developed method was validated and the content of SY was found to be below the levels recommended by WHO/FAO (0-4 mg/kg/b.w.). The employed method was found to be not suitable for solid dosage forms.

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Conflicts of Interest

The authors declare no conflict of interest.

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