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Development of an UV spectroscopic method for Capsaicin quantification in dosage form and in a bulk formulation

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Abstract: A proper procedure must be created to make sure that capsaicin, whether in dosage form or in bulk, can be identified. The method development makes sure that the amount of a capsaicin can be simply determined. To determine validation parameters like accuracy, precision, LOD, LOQ, recovery study and range for capsaicin. In the present study a proper UV Spectroscopic method was developed and validated for determination of capsaicin. UV Spectrophotometric method was done at 280nm and samples were prepared with Methanol. The linearity demonstrated a correlation coefficient of 0.9922 various validation parameters like accuracy, precision, LOD, LOQ, recovery study and range were found to be within the specified range. The proposed method was simple, rapid, precise, accurate and sensitive and can be used for routine analysis of capsaicin.

Keywords: Capsaicin, UV method, Validation, Spectrophotometry, Stress degradation, Calibration curve.

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1. INTRODUCTION

Capsaicin (8-methyl-N-vanillyl-6-nonenamidepossesses any anti-inflammatory effects equivalent to non-steroidal anti-inflammatory analgesics (NSAIDS). It is typically used in dosages between 0.025 % and 0.1 % as an analgesic in ointments and dermal patches to relieve pain. It can be administered topically in cream form to temporarily relieve backache, arthritis, mild muscle and joint aches, as well as strains and sprains [1] (figure 1).

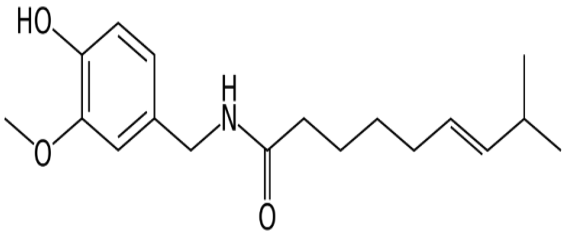

Fig 1: Structure of capsaicin

When compared to other techniques, the UV spectrophotometric method is highly straightforward, rapid, economical, and it enables the accurate determination of pharmaceuticals. The review of the literature for the UV spectrophotometric method showed fairly intricate techniques that employed bands of the visible spectrum employing complexometry, derivative assistance, and interpolation on the calibration curve. [2-3]. The goal of this work was to develop & validate a novel UV spectrophotometric method that can be more affordable & less complicated than currently used techniques as well as other approaches that have been published. The UV spectrophotometric method is less complicated than the others because it does not require derivative or chemometric support. The fact that this method uses its own dissolve media as a diluent also makes it applicable to dissolution studies [2-3] The most important step in the development of any drug, whether it be in combination or in bulk, is analysis. A proper procedure must be created to make sure that any of the drug, whether in dosage form or in bulk, can be identified. The method development makes sure that the amount of a specific drug can be simply determined. The validation parameters demonstrate the developed method's accuracy, precision, reproducibility and show that it may be utilised for routine evaluation of capsaicin [2-4]. To develop and validate an analytical method by using UV-VIS Spectrophotometry for the estimation of capsaicin is a purpose of this work. Stress testing must be completed in order to clarify the intrinsic stability properties of the active substance, conferring to the International Conference on Harmonization (ICH) regulation eligible stability assessment of novel drug substances & goods. The purpose of this work was to use the proposed approach to conduct stress degradation experiments on capsaicin. [12]

2. MATERIALS AND METHODS

Instrumentation is found to be with 10mm matched quartz cells a UV-Visible Spectrophotometer (UV-1800 SHIMADZU) was used for spectrophotometric method. On electronic balance all balancing were done (Model Shimadzu AUW-220D). In reagents and chemicals**, c**apsaicin gift sample was received from Srihit lab Pvt Ltd, Hyderabad, India. All solvents and chemicals used were of HPLC Grade or analytical grade, purchased from Merck Specialities Pvt Ltd Mumbai.

2.1. Standard Stock Solution preparation

Standard stock solution of capsaicin was prepared by exactly deliberating 10 mg of the drug, and dissolved in methanol and the volume was made up to 100ml to obtain concentration of 100 µg/ml i.e. stock solution [5-6]

2.2. Determination of Analytical Wavelength

1 ml of the standard stock solution was pipette out into a 10 ml volumetric flask. The capacity was made up to 10ml through methanol. The final solution contains 10 µg/ml and between 200-400 nm this final solution was scanned [5-6].

2.3. Preparation of Calibration Curve

Diluent of 0.5, 0.7, 1.0, 1.3, 1.5, 1.8 & 2.0 ml ratio of stock solutions were transferred to a sequence of 10ml volumetric flasks, and volume made up to the spot with methanol. The serial dilutions in the range of 5, 7, 10, 13, 15, 18 and 20 µg/ml were prepared. The absorbance was measured at λ_{max} 280nm [5- 9].

2.4. Linearity and Range

At 5-20µg/ml concentrations the linearity of the response of the drug was confirmed. The absorbance versus concentration data were plotted to create the calibration curve, which was then subjected to a linear regression analysis. The equation of the calibration curve for capsaicin was obtained [5-9].

2.5. Precision

Recovery experiments determined the method's accuracy. A calculation of the %age recovery was done after each dilution was performed three times. Studies on intra-day and inter-day fluctuation showed that the procedure is accurate [5- 9].

2.6. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD & LOQ were calculated with the help of following equations;
LOD = 3.3 б/ S and
LOQ = 10 б/S
Where S = Slope of the calibration curve
б = residual standard deviation.

2.7. *Recovery Study*

Recovery studies were used to examine the method's accuracy. Three levels of recovery were carried out: 80, 100, and 120 % of the capsaicin standard concentration. For each recovery level, the recovery dilutions were created using the aforementioned technique. The solutions were then examined, and using the calibration curve, percentage recoveries were calculated [7-11].

2.8. *Robustness*

The robustness of the approach was assessed by running the study at two different temperatures, namely room temperature and 25°C in a stability chamber. The results were expressed as a % RSD after the individual 20 g/ml absorbance was noted.

2.9. *Ruggedness*

The method was assessed by having various analysis by various analyst and the equivalent absorbance of 20 µg/ml was noted. The outcome was shown as % RSD.

3. RESULTS

3.1. *Analytical Wavelength*

The wavelength for capsaicin was determined to be 280 nm as a result of the extreme absorption being at that wavelength (figure 2).

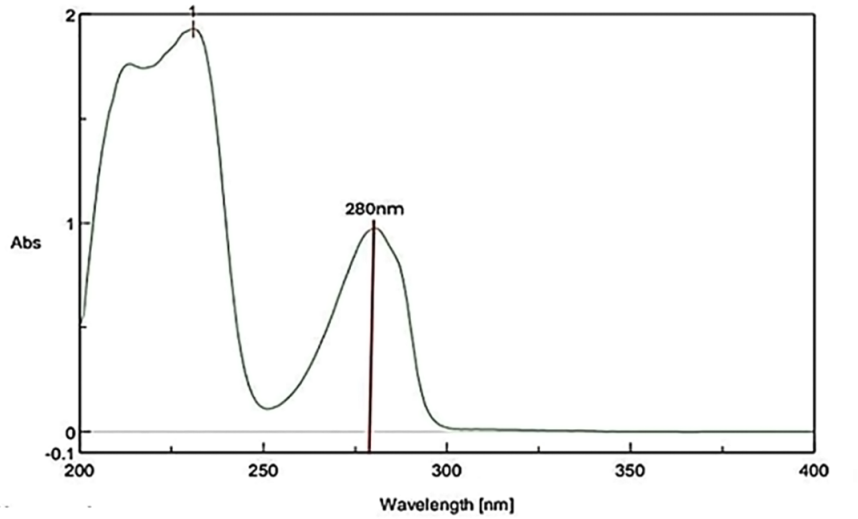

Fig 2: UV Spectrum of capsaicin at 280 nm

3.2. *Calibration Curve*

Concentration vs. Absorbance results were plotted, and the method was found to be linear over the produced concentration range using the standard equation y=0.0429x+0.0036. The regression value was discovered to be 0.9992. It was discovered from the calibration data that the regression coefficient was less than 1, which is within the bounds of Beer Lamberts' law (figure 3) (table 1) (table 2).

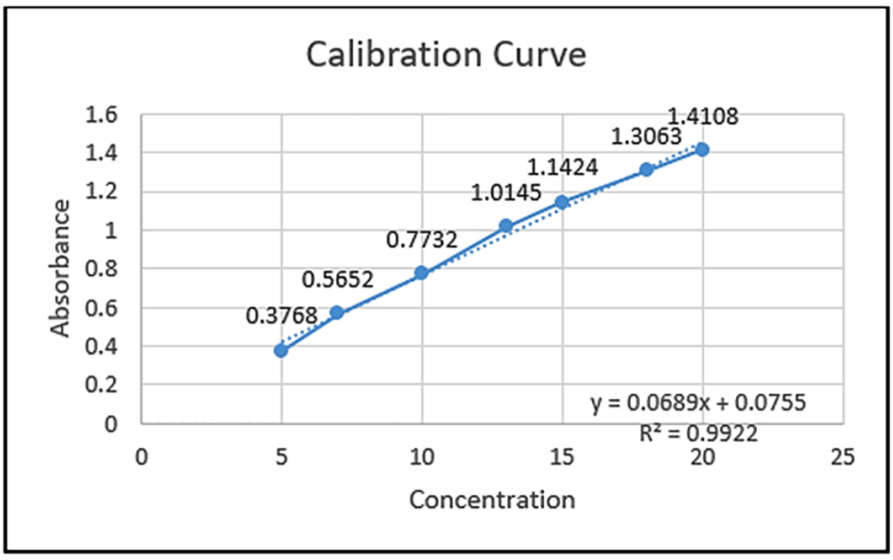

Fig 3: Standard calibration curve of capsaicin

Table 1: Calibration curve data		
SN	Concentration (ppm)	Absorbance
1	5	0.3768
2	7	0.5652
3	10	0.7732
4	13	1.0145
5	15	1.1424
6	18	1.3063
7	20	1.4108

Table 2: Optical characteristics of capsaicin	
Parameter	Result
Beer's law limit (µg/ml)	5- 20 µg/ml
Correlation coefficient	0.9922
Regression equation (Y*)	0.0689x + 0.0755
Slope (a)	0.0689x
Intercept (b)	0.0755

3.3. Precision

For capsaicin, the method's accuracy was estimated. In the same study facility, the method's repeatability and reproducibility, or intra-day and inter-day precision, were assessed. RSD obtained on average was 0.89 % and 1.183 %, respectively (table 3 & table 4). The procedure was confirmed to be precise in terms of reproducibility and repeatability.

Table 3: Inter-day precision studies for capsaicin						
Conc. (µg/ml)	Absorbance (nm)			Mean	SD	% RSD
	Day 1	Day 2	Day 3			
5	0.373	0.364	0.365	0.367333	0.004028	1.09
7	0.565	0.559	0.554	0.559333	0.004497	0.84
10	0.771	0.776	0.785	0.777333	0.005793	0.74
					Average of % RSD= 0.89%	

Table 4: Intra-day precision studies for capsaicin						
Conc. (µg/ml)	Absorbance (nm)			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
5	0.376	0.369	0.361	0.368667	0.006128	1.66
7	0.565	0.551	0.559	0.558333	0.005735	1.02
10	0.773	0.757	0.762	0.764	0.006683	0.87
					Average of % RSD=1.183%	

3.4. Accuracy (Recovery study)

Recovery studies were used to examine the method's accuracy. Three levels of the usual capsaicin concentration 80, 100, and 120 % were used for the recovery. For each recovery level, three samples of each concentration were created. The solutions were examined, and using the calibration curve, the % age recoveries were computed (table 5).

Table 5: Accuracy study of capsaicin bulk drug					
Preparation number: Conc. (%)	Concentration (µg/ml)		Drug found (µg/ml)	% recovery	Mean (%)
	Amount	Amount added			
S_1:80%	1.6	1.28	1.270	99.2	99.9
S_2:80%	1.6	1.28	1.281	100.1	
S_3:80%	1.6	1.28	1.285	100.4	
S_1:100%	1.6	1.60	1.630	101.9	100.0
S_2:100%	1.6	1.60	1.590	99.3	
S_3:100%	1.6	1.60	1.580	98.7	
S_1:120%	1.6	1.92	1.924	100.3	99.8
S_2:120%	1.6	1.92	1.926	100.3	
S_3:120%	1.6	1.92	1.900	98.9	

3.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The lowest amount of analyte is limit of detection which is detectable but cannot be effectively quantified; limit of quantification is the lowest concentration that can be measured. It was determined that the LOD and LOQ stayed 0.029 g/ml and 0.089 g/ml, correspondingly.

3.6. Validation Parameters

All validation parameters as reported in table no.6 were found to be within the preferred range which shows that the method was found to be reproducible with respect to all the validation parameters and can be a helpful tool for routine capsaicin assessment (table 6).

Table 6: Summary of validation of capsaicin

SN	Parameter	Result
1	Linearity indicated by correlation coefficient	0.9922
2	Linear regression equation	0.0689x + 0.0755
3	Range	5 µg/ml – 20 µg/ml
4	Intra-day Precision (%RSD)	1.18%
5	Inter-day Precision (%RSD)	0.89%
6	Limit of Detection	0.029 µg/ml
7	Limit of Quantification	0.089 µg/ml
8	Robustness indicated by % RSD	0.177%
9	Ruggedness indicated by % RSD	0.4188%

3.7. Stress degradation by hydrolysis under various conditions

3.7.1. Acidic degradation

2 ml of capsaicin stock solution was added to and mixed with 3 ml of each of the following concentrations of hydrochloric acid: 0.1N, 0.5N, 1N, 1.5N, 2N, and 2.5N in each of the six volumetric flasks. Those volumetric flasks were maintained at room temperature for 3 hours. After 3 hours, the solution was neutralised, methanol was added to carry the capacity up to 10 ml, & the dilution method was completed to achieve the desired concentration (20 g/ml). 3 hours had passed, and the deterioration in the 1N hydrochloric acid had already begun**.** The % purity of standard drug without stress condition is 70.45 % and % purity of drug after acidic condition 57.55%. Therefore, 12.9% drug has been degraded in acidic condition (figure 4).

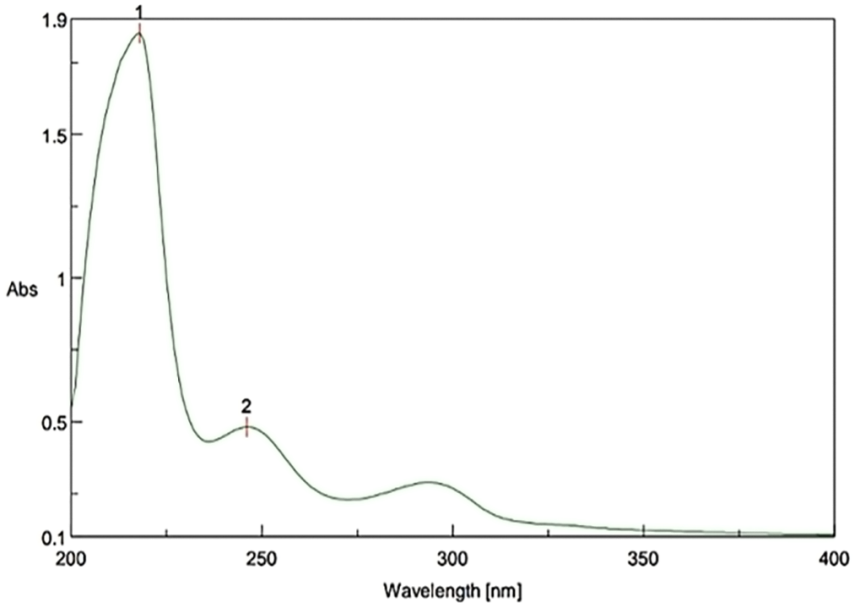

Fig 4: Acidic degradation of sample of capsaicin.

3.7.2. Alkaline degradation

2 ml of the capsaicin stock solution and 3 ml of each of the following solutions were added to the six distinct volumetric flasks: 1N, 1.5N, 2N, 2.5N, 3N, 3.5N sodium hydroxide. 3 hours were spent keeping those volumetric flasks at room temperature. After three hours, the solution was neutralised, diluted with methanol to bring the volume to 10 ml, and the dilution process was completed to obtain the desired concentration (20 g/ml). The deterioration of the 0.5 N sodium hydroxide was noticed after the 3-hour time period was complete. The % purity of standard drug without stress condition is 70.45% and % purity of drug after alkali condition 63.15%. Therefore, 7.3% drug has been degraded at alkaline condition (figure 5).

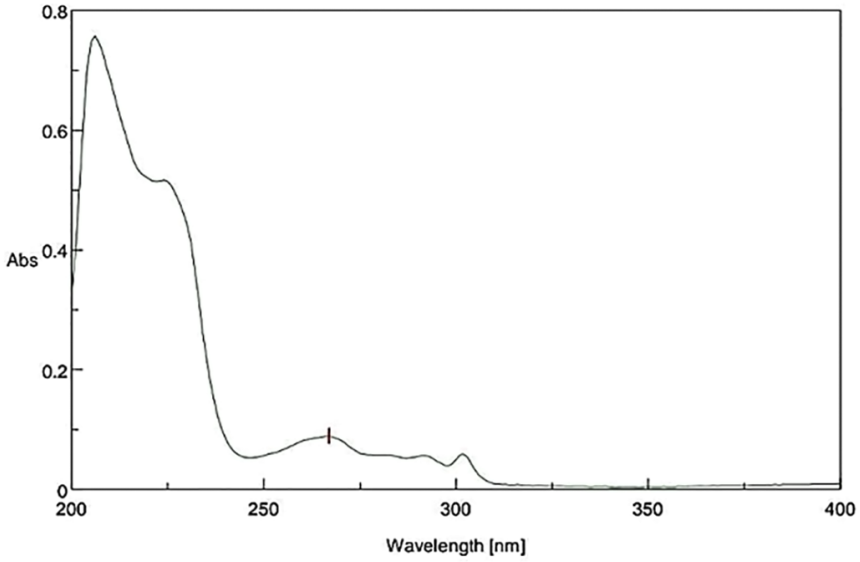

Fig 5: Alkaline degradation of sample of capsaicin.

3.7.3. Photolytic degradation

For 24 hours, a sample of capsaicin was exposed to UV radiation. A volume of 10 ml was created after dissolving a 20 mg sample in methanol. Methanol was used to dilute this solution appropriately (20 g/ml), and this solution was then taken in a cuvette for the UV investigation (figure 6).

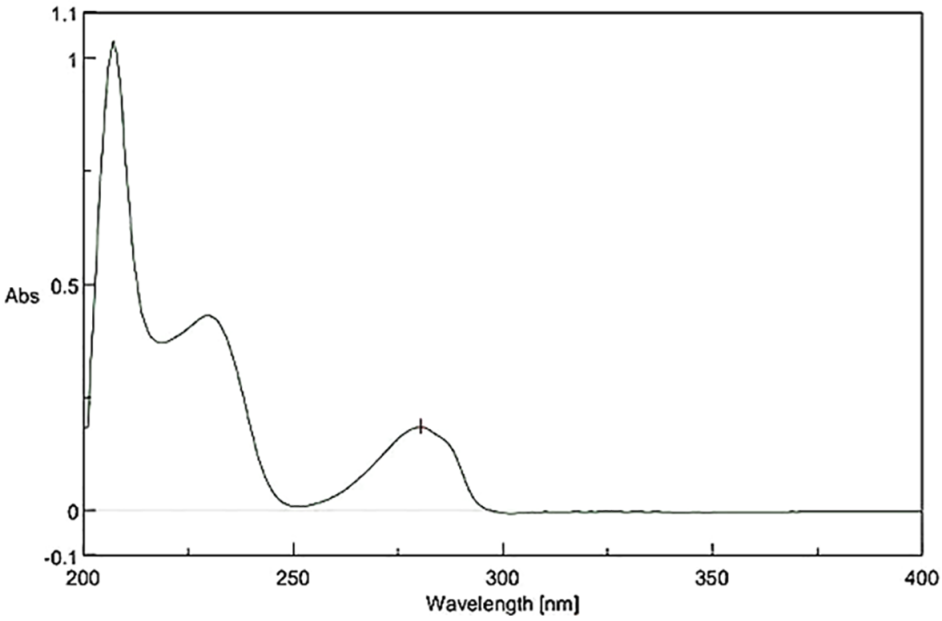

Fig 6: Photolytic degradation of sample of capsaicin.

The % purity of standard drug without stress condition is 70.45 % and % purity of drug after photolytic condition 69.04%. Therefore, 1.05% drug has been degraded in photolytic condition.

3.7.4. Thermal degradation

A sample of capsaicin was collected in a petri plate and heated in an oven to 60°C for 48 hours. After removing the sample from the oven and letting it cool for an hour, following an hour, methanol was used to dilute 20 mg of the sample to a final amount of 10 ml. Dilutions were made from this solution to obtain the required concentration (20 g/ml), and this solution was then taken in a cuvette for UV-Vis investigation. The % purity of standard drug without stress condition is 70.45% and % purity of drug after thermal condition 67.65%. Therefore, 2.8% drug has been degraded in thermal condition (figure 7).

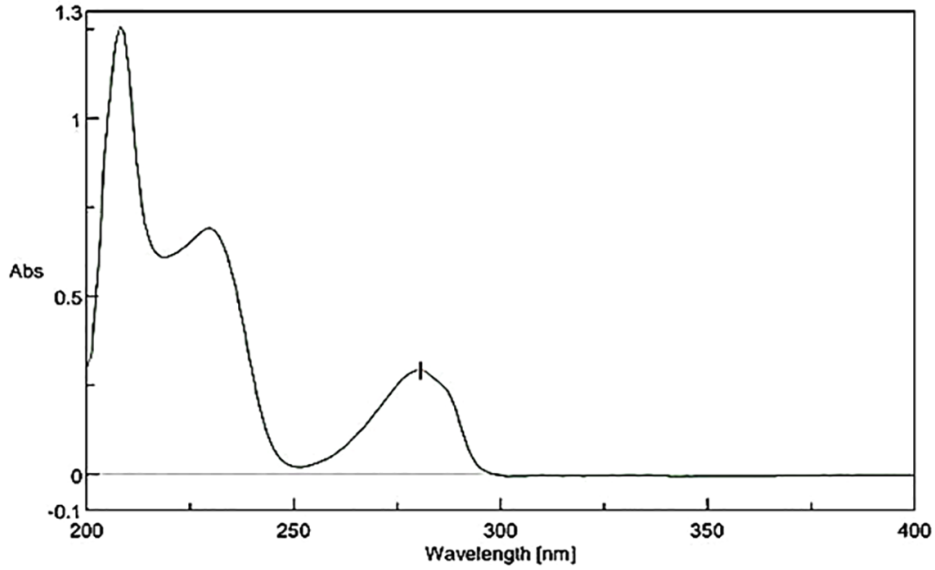

Fig 7: Thermal degradation of sample of capsaicin.

3.7.5. Oxidative degradation

In six different volumetric flasks 2 ml of stock solution of capsaicin was added and 3 ml of 1%, 3%, 6%, 20%, 30% v/v of H_2O_2 was added in every volumetric flask respectively. The volumetric flask was kept for 3 hours. After every 1 hr time break dilution was carried out to achieve the appropriate concentration (20 µg/ml). In the 6% v/v of hydrogen peroxide degradation was observed**.** The % purity of standard drug without stress condition is 70.45% and % purity of drug after oxidative condition 69.0%. Therefore, 1.45% drug has been degraded in oxidative condition (figure 8).

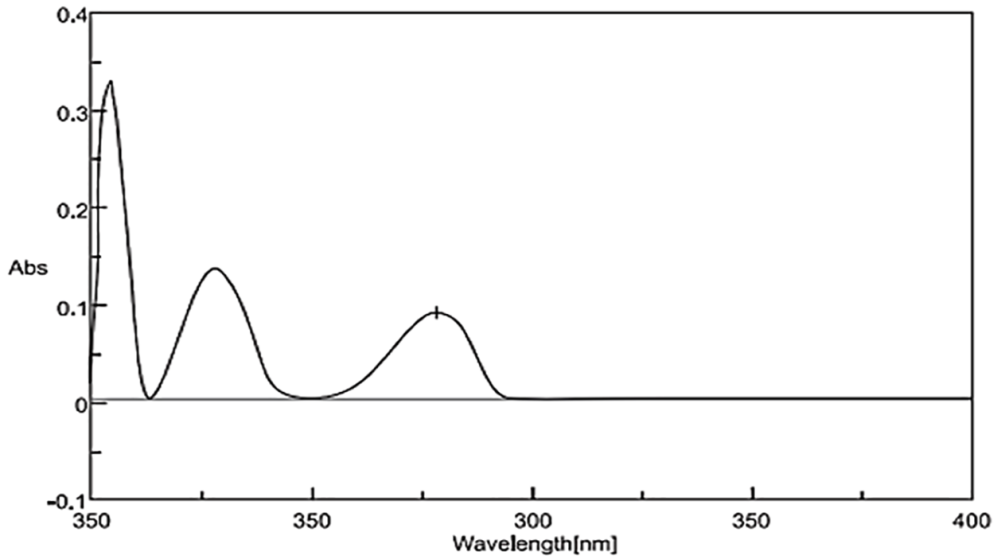

Fig 8: Thermal degradation of sample of capsaicin.

4. RESULTS & DISCUSSION

For capsaicin a viable UV spectroscopic method was developed and validated. The process was found to be linear over the prepared concentration range with the standard eq. y = 0.0689x+ 0.0755. The regression coefficient was 0.9922. Within the bounds of Beer Lamberts' law, the regression coefficient derived from the calibration data was less than 1. In the same labs the repeatability (intra-day precision) of the method and reproducibility (inter-day precision) was evaluated. LOD and LOQ were determined to be 0.029 g/ml and 0.089 g/ml, correspondingly. The % RSD obtained were 1.18% and 0.89%, respectively, showing that the procedure had good repeatability. Stress degradation study was also studied for said research work (table 7).

Table 7: Summary of result of stress degradation studies

Stress condition	Time	Observation	Concentration of capsaicin degraded (µg/ml)	% degradation
Acidic Degradation	Reflux 1 hours	⌊ max shifted	2.58	12.9
Alkali Degradation	Reflux 1 hours	⌊ max shifted	1.46	7.3
Photo degradation	24 hours	No λ max shifted	0.21	1.05
Thermal degradation	48 hours	No λ max shifted	0.56	2.8
Oxidative degradation	RT 1 hours	No λ max shifted	0.29	1.45

5. CONCLUSION

An appropriate UV Spectrophotometric technique was developed & validated for the determination of capsaicin in accordance with ICH recommendations. It was shown above that the proposed method was linear, accurate, reproducible, repeatable, precise, selective and inexpensive proving the credibility of the method. Furthermore, only one solvent was utilised for the period of the experiment, and it was determined that there was no excipient interference with the procedure. Hence, the proposed technique was effectively used to conduct repetitive analysis of capsaicin.

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7. AUTHORS CONTRIBUTION STATEMENT

Dr Atul Baravkar was involved in study conception and design, Dr Vitthal Chopade and Ms Sneha Dhonde were contributed for analysis and interpretation of results, Trupti Mandhare, Pooja Kashid and Ganesh Phadtare were contributed for draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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