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Validation of UV spectrophotometric method for the analysis of capsaicin in ethanol

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ABSTRACT

Method validation of gift sample capsaicin was carried out in calibrated double beam UV/visible spectrophotometer (Shimazdu UV-01800) using ethanol as diluent phase. Though, Capsaicin showed maximum absorbance peaks at 227nm and 280 nm, a 280nm wavelength was considered as absorbance peak as it was very strong at this wavelength. The correlation coefficient (R^2) and linear regression equation were found to be 0.9924 and Y= 0.0155x -0.0338 respectively in linear- calibration curve. Further, this method was validated in terms of Linearity, Accuracy, Precision, Limit of Detection (LOD), Limit of Quantification (LOQ), repeatability and robustness as per ICH guidelines.

Keywords: Capsaicin, method validation, UV/visible spectrophotometer, linear-calibration curve, ICH-guidelines.

INTRODUCTION

Capsaicin (C18H27NO3),8-methyl-N-vanillyl-6-nonenamide, is a unique alkaloid found primarily in the fruit of the Capsicum genus and thus provide spicy flavor, has molecular weight 305.40 g/mol with highly soluble in ethanol (30mg/ml at least) and olive oil, that bounds to transient receptor potential vanilloid 1 (TRPV1) mainly expressed in sensory neurons, containing a heat-sensitive subunit responsible for the burning sensation caused by capsaicin[1]. Capsaicin triggered responses are used to relieve different kind of pain as analgesic to its clinical therapeutics uses in treating various diseases like obesity, diabetes, cardiovascular conditions, cancer, airway diseases, itch, gastric, and urological disorders[2].Although , HPLC validation methods are more frequently available[3] ,an alternative effective UV spectrophotometric method for analysis of capsaicin has been developed as per ICH guidelines[4] and validated here which is more ease for accurate determining in less commercialized laboratory set-up.
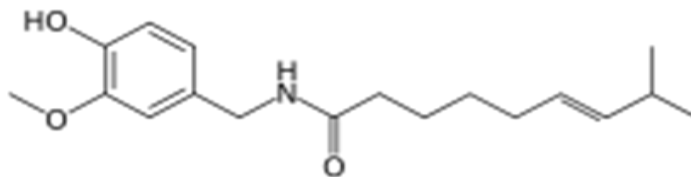
Fig 1: Structure of capsaicin [1]

Materials and methods
Materials

Calibrated double beam UV/visible spectrophotometer (Shimazdu UV-01800) having spectral bandwidth 1nm and 1.0 cm matched quartz cell was used for UV method analysis for capsaicin. A standard capsaicin was obtained as gift sample from Prime Pharmaceutical Pvt. Ltd, Birgunj,Nepal. Ethanol (95%v/v), which was used for dilution of capsaicin, was of analytical grade and was readily available in our lab.

Preparation of standard stock solution

10mg standard capsaicin was taken in 100ml volumetric flask.50ml ethanol was added and sonicated for 5 minutes then diluted by ethanol up to the mark of volumetric flask to prepare final strength of solution i.e. , 100μg/ml.

Scanning of wavelength in ethanol

Appropriately, 4ml of standard stock solution was taken in 10ml volumetric flask, diluted up to the mark by ethanol to give final strength of solution i.e. , 40μg/ml and resulting solution was scanned in UV range(200nm-400nm). Maximum absorbances were seen at 227nm and at 280 nm (Figure-2), though 280nm wavelength was considered, considering UV cutoff effect of ethanol at 227nm.
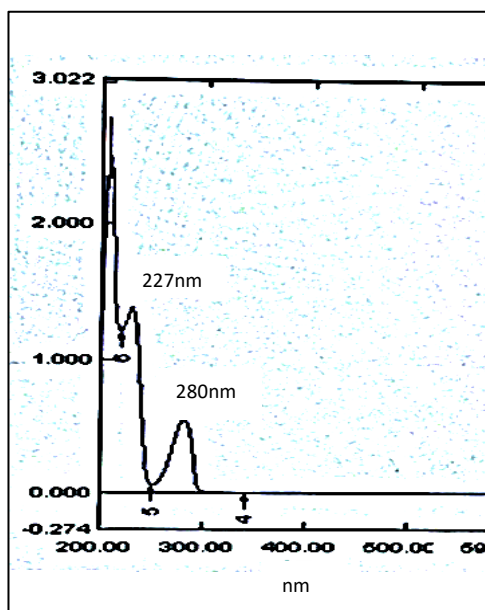

Fig 2: UV spectrum of capsaicin scanning at (200-400) nm

Method validation

Linearity study

Different concentrations of standard capsaicin, i.e., 20 μg/ml, 30 μg/ml, 40 μg/ml, 50 μg/ml, 60 μg/ml were made by taking series of aliquots 2ml,3ml,4ml,5ml and 6ml from stock solution on 10ml volumetric flask separately and Each volumetric flask was diluted by ethanol(95%v/v purity) up to mark 10 ml. Now, absorbances were seen at 280nm and calibration curve was plotted as shown in Fig 3.

calibration curve

$y = 0.0155x - 0.0338$
$R^2 = 0.9924$
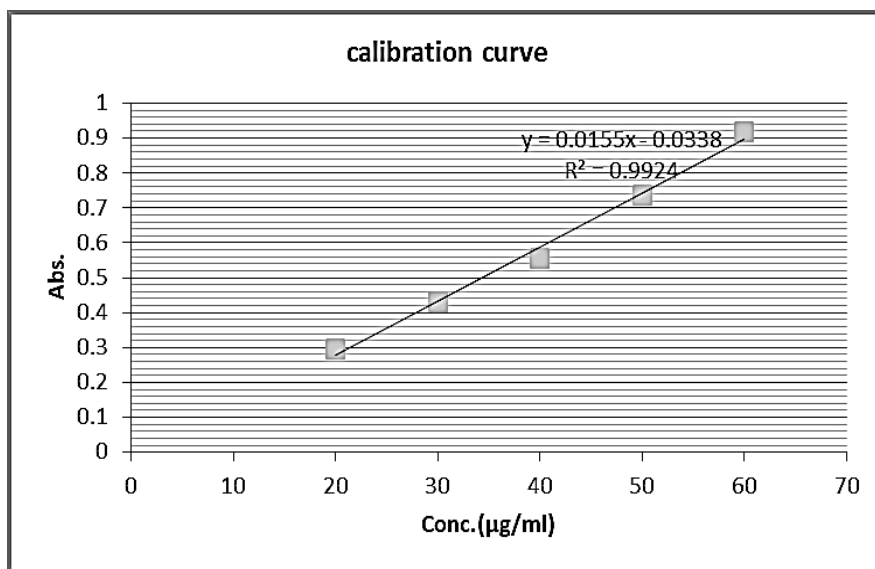
Fig 3: Calibration plot for standard capsaicin

Accuracy study

A known amount of standard stock solutions were added at different levels (n=3) i.e. 80%, 100% and 120% to the pre-analyzed sample solutions and solutions were reanalyzed by purposed method.

Precision study

The intraday and interday variations studied were performed. For intraday precision at same day, analysis of 40 μg/ml, 50 μg/ml, 60 μg/ml sample solutions were done three times (n=3) and for interday precision ,analysis of samples 40 μg/ml, 50 μg/ml, 60 μg/ were done by same analyst once for 3 days.

Limit of detection study

It was calculated by,

$$\%LoD = \frac{3.3 \times \text{Standard deviation of response}}{\text{slope of calibration curve}}$$

Limit of quantification study

It was calculated by,

$$\%LoQ = \frac{10 \times \text{Standard deviation of response}}{\text{slope of calibration curve}}$$

Repeatability study

Repeatability was determined by analyzing 50 μg/ml standard solution of capsaicin in ethanol for six times (n=6).

Robustness study

At same environmental and operation condition, a sample solution strength 30 µg/ml was measured at small change in wavelengths i.e. 279nm,280nm and 281nm for six times(n=6) each.

Results and discussion

Linearity study

A linear graph of absorbance vs. standard concentration (µg/ml) from figure 3 showed correlation coefficient (R^2) value was 0.9924 with regression equation Y= 0.0155x -0.0338. Thus, regression analysis for the linearity showed very good correlation between absorbance and concentration at 280nm showing that the method of analysis of capsaicin sample by UV-visible spectrophotometer was validated as per ICH guidelines. The responses of concentrations at 280nm were shown (Table 1).

Table 1: Linearity result for UV

Concentration(µg/ml)	Absorbance
20	0.295
30	0.430
40	0.555
50	0.735
60	0.918

Accuracy study

The accuracy result for UV (Table 2) showed that the %RSD calculated for different reanalyzed concentration levels, i.e. 40 µg/ml, 50 µg/ml, 60 µg were less than 2 indicating that method was accurate as per ICH guidelines.

Table 2: Accuracy result for UV

Pre-analyzed sample solution(µg/ml)	Amount of drug added (µg/ml)(n=3)	Recovery amount[a] (µg/ml)(n=3)	% Recovery	% RSD
50	0	49.98	99.96	0.07
	40	90.29	100.32	0.58
	50	100.20	100.20	1.06
	60	110.29	100.26	1.01
a= average of three replicates				

Precision study

The Precision result for UV (Table 3) showed that the %RSD calculated for different intraday precision and interday precision were less than 2 indicating that method was precised as per ICH guidelines.

Table 3: Precision study for UV

Analyte	Concentration (µg/ml)	Intraday precision[a](n=3)		Interday precision[a](n=3)	
		Conc. found	%RSD	Conc. found	%RSD
capsaicin	40	39.74	1.05	40.03	0.83
	50	50.07	0.67	50.02	1.32
	60	60.70	0.68	60.33	0.82
a= average of three replicates					

Limit of detection and limit of quantification study

The Limit of Detection and Limit of Quantification study were found 24.03μg/ml and 72.83 μg/ml respectively.

Repeatability study

The Repeatability result for UV (Table 4) showed that the %RSD was less than 2 indicating that method was able to reproduce as per ICH guidelines.

Table 4: Repeatability study for UV

Concentration(μg/ml)	Absorbance	%RSD
50	0.735	
50	0.730	
50	0.742	0.92
50	0.748	
50	0.733	
50	0.733	

Robustness study

The absorbances of six replicates of capsaicin solution strength 30μg/mL measured by wavelengths, i.e.280± 1nm showed that each % RSD is less than 2 (Table 5).

Table 5: Robustness study for UV

Concentration(μg/ml)	Sample number	279nm	280nm	281nm
30	1	0.430	0.433	0.431
	2	0.427	0.430	0.430
	3	0.430	0.432	0.431
	4	0.427	0.429	0.429
	5	0.428	0.432	0.427
	6	0.430	0.431	0.429
	%RSD	0.35	0.34	0.35

CONCLUSION

The purposed UV/Visible spectrophotometric analytical method for determination of capsaicin was accurate, precise, sensitive and thus reproduced easily. This validated method can be used for quality control of capsaicin related pharmaceuticals products easily and thus in more cost effective manner than readily available High Performance Liquid Chromatography (HPLC) method for analysis.

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