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DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTRO-PHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TACROLIMUS AND BERBERINE HYDROCHLORIDE

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Keywords:

Tacrolimus, Berberine hydrochloride, First order derivative spectroscopy, Zero crossing point, Correlation coefficient, ICH guidelines

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ABSTRACT: A simple UV spectrophotometric method using the first order derivative technique was developed for the simultaneous estimation of tacrolimus and berberine hydrochloride in nanoparticles. The tacrolimus and berberine hydrochloride stock solutions were prepared methanol and scanned in UV- region for first-order derivative spectrum. The zero-crossing point for tacrolimus and berberine hydrochloride was 369 nm and 263 nm, respectively. Linearity was established over the concentration range of 0.5-2.5 µg/ml and 5-25 µg/ml for tacrolimus and berberine hydrochloride with correlation coefficient (r^2) value 0.9933 and 0.9796respectively. The method was validated according to ICH guidelines for validation parameters like accuracy, precision, the limit of detection, and the limit of quantification. They found to be within limits. The method was successfully applied for the quantitative estimation of tacrolimus and berberine hydrochloride in nanoparticles.

INTRODUCTION: Tacrolimus (FK 506) is a drug used for the treatment of Rheumatoid Arthritis (RA). It exerts its immunosuppressive effects by the inhibition of calcineurin, leading to interference with T-cell activation and suppressing inflame-matory cytokines, healing of joint inflammation, and reducing bone cartilage destruction, improvement of functional status, and relief from arthritic pain. This drug is associated with some major dose-related adverse drug reactions (ADRs) like nephrotoxicity, infectious and malignant complications, neurotoxicity, diabetogenic and hypertension.

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Tacrolimus is BCS class II drug having low aqueous solubility and low bioavailability due to its metabolism through the gut and also due to its incomplete absorption through GIT [1]. Berberine (BBR), extracted from Phellodendron Chinese and Coptis Root, has aroused wide interest due to its variety of pharmacological activities *i.e.*, anti-cancer, anti-inflammatory, anti-diabetic, and anti-hyperglycemic in recent years. BBR is an effective and nontoxic agent in the clinic.

However, similar to some other herbal products, the further development and clinical application of BBR has been limited by its poor aqueous solubility and low gastrointestinal absorption [2]. Tacrolimus and berberine are available in a single dosage form but not in combination therapy in the market. Fixed-dose combination therapy of tacrolimus and berberine is indicated for the treatment of Rheumatoid Arthritis (RA) and different types of Cancer.

It has been hypothesized that adverse effect of TAC can be reduced upto some extent if a very low dose of tacrolimus is combined with Berberine (BBR) which may compensate for the effect of tacrolimus and reduce its ADRs. Literature survey reveals various analytical methods for the estimation of TAC and BBR individually using UV spectrophotometry, HPLC, and HPTLC. Moreover, many methods were reported for the estimation of TAC or BBR along with other drugs in the combined formulation. However, the development of simultaneous estimation of TAC and BBR in combined dosage form has not yet been reported by any method. Hence, this manuscript is the first to describe the development and validation of some simpler, sensitive, precise, accurate, and cost-effective UV spectroscopic methods for the simultaneous determination of TAC and BBR in Albumin loaded nanoparticles. Proposed methods possess several advantages, which are as follows; methods describe very simple standard, and sample preparation procedure, wide concentration range with high sensitivity, and all the developed methods were validated as per ICH guidelines.

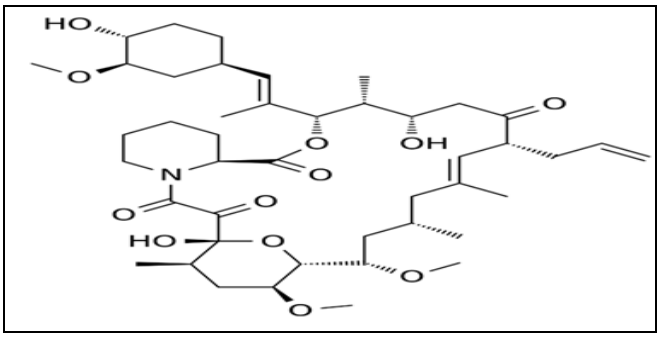

FIG. 1: CHEMICAL STRUCTURE OF TACROLIMUS

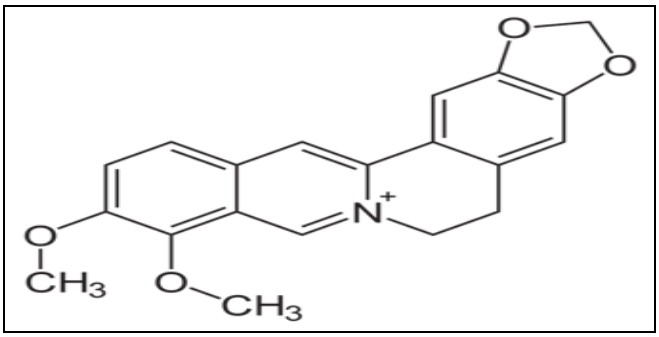

FIG. 2: CHEMICAL STRUCTURE OF BERBERINE

MATERIALS AND METHODS:

Chemicals and Reagents: TAC was procured from Centurion laboratories private limited (Vadodara, India). While BBR was obtained from Yucca enterprises (Mumbai, India). HSA was purchased from Xi'an harmonious natural biotechnology co., LTD (China).

Apparatus: Shimadzu double beam UV visible spectrophotometer (UV-1800, UV Probe, Shimadzu Corporation, Kyoto, Japan) with matched quartz cell of 1 cm path length was used throughout the experiment. Highly sensitive electronic balance Adventurer Pro AVG264C, Ohaus Corporation, Pine Brook, NJ, USA was used for weighing purposes.

Preparation of Standard Solution: Stock solution of TAC and BBR were prepared individually by weighing accurately 10 mg of standard drugs and transferred to a 10 ml volumetric flask separately. Standard drugs were diluted to 10 ml with methanol to get the concentration of the drugs 1000 µg/ml. Further, dilutions were made to get the required concentration with methanol.

Preparation of Sample Solution: 4 mg of formulated nanoparticles was added to 10 ml of methanol to precipitate protein, followed by centrifugation at 3000 rpm for 5 min. After suitable dilution, the supernatant was scanned in the UV region of 200-400 nm, and spectrums were recorded and were converted into first derivative spectra. The conc. of TAC was determined at 263nm, and BBR was determined at 369 nm by using the regression equation of calibration curve.

Procedure: First Derivative (zero crossings) method the normal UV spectra of TAC and BBR were transformed into first derivative spectra. Based on the spectral pattern and zero-crossing points, first DR (derivative spectroscopic) method was chosen for the study. First derivative spectra showed typical zero-crossing points at 369 nm for TAC and 263 nm for BBR, applying 2 nm as wavelength interval ($\Delta\lambda$) and 1 as scaling factor.

After assessing overlain spectra, 369 nm and 263 nm were selected for further studies. A calibration curve was plotted for both TAC and BBR in the concentration range of 0.5-2.5 µg/ml and 5-25 µg/ml, respectively. Results were subjected to regression analysis by the least square method to determine the values of the slope, intercept, and correlation coefficient.

Method Validation: The method developed was validated for linearity, precision, accuracy, specificity, ruggedness, robustness, the limit of detection, the limit of quantification, and robustness according to the ICH Guidelines.

Linearity and Range: The linearity of an analytical method is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample. The range of the analytical method is the interval between upper and lower concentration (amounts) of analyte that have been demonstrated with a suitable level of precision, linearity and accuracy [3, 4].

Linearity was established over the concentration range of 0.5-2.5 µg/ml and 5-25 µg/ml for tacrolimus and berberine hydrochloride. The absorbance of these solutions was measured at 369 nm, and 263 nm against the solvent blank, respectively, and D1 (first-order derivative) absorbance values were recorded. A graph of concentration versus absorbance was plotted, and a correlation coefficient (r^2) was reported.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value.

The quantification limit of an individual analytical procedure is the lowest amount of analyse in a sample which can be quantitatively determined with suitable precision and accuracy. The sensitivity of proposed method for measurement of TAC and BBR was estimated in terms of LOD & LOQ determined using the standard deviation of the response and slope method [3-5].

LOD= 3.3 × Standard Deviation / Slope

LOQ= 10 × Standard Deviation / Slope

Precision: The precision of an analytical procedure defines the degree of closeness of agreement between a series of measurements obtained from multiple samplings of the homogenous sample under prescribed conditions. The precision of the method was reported as RSD% at different levels-repeatability, Intra-day precision, and Inter-day precision [4-6]. Repeatability was evaluated by the analysis of three replicates of 2.5 µg/ml of TAC and 25 µg/ml of BBR for checking the variation of results on the same day. The intra-day and inter-day precision of the proposed method was determined by analyzing samples 0.5 µg/ml, 1.5 µg/ml, and 2.5 µg/ml for TAC and 5 µg/ml, 15 µg/ml, 25 µg/ml for BBR, three replicates of each sample as a batch in a single assay on the same day and three consecutive days for inter-day precision.

Accuracy: Recovery studies were carried out by measuring the peak amplitude of derivative spectra at 369 nm and 263 nm wavelength of the added standard drug to pre-analyzed sample solution at three different levels: 50, 100 and 150% to check the accuracy of the method [4, 6].

The resulting solutions were reanalyzed, and % recovery was calculated.

% Recovery = (Amount of drug found after addition of standard drug – Amount of drug found before the addition of standard drug) / (Amount of standard drug added) × 100

RESULTS AND DISCUSSION:

Linearity and Range: Linearity and range of the method were checked by analyzing all the standard mixture solutions and measuring the peak amplitude of the derivative spectra at their respective wavelength.

Results were subjected to regression analysis by the least-squares method to calculate the values of the slope, intercept, and correlation coefficient. The linearity of the method was found in the range of 0.5-2.5 µg/mL of TAC and 5-25 µg/mL of BBR.

TABLE 1: PEAK AMPLITUDE TABLE OF STANDARD MIXTURE SOLUTION

S. no.	TAC at 263 nm		BBR at 369 nm	
	Conc. (µg/mL)	Peak Amplitude Mean ± SD	Conc. (µg/mL)	Peak Amplitude Mean ± SD
1	0.5	0.0092 ± 0.0001	5	0.021 ± 0.001
2	1.0	0.0155 ± 0.0003	10	0.0326 ± 0.0001
3	1.5	0.0197 ± 0.0002	15	0.0439 ± 0.0001
4	2.0	0.0269 ± 0.00001	20	0.0683 ± 0.0002
5	2.5	0.0337 ± 0.0002	25	0.815 ± 0.0001

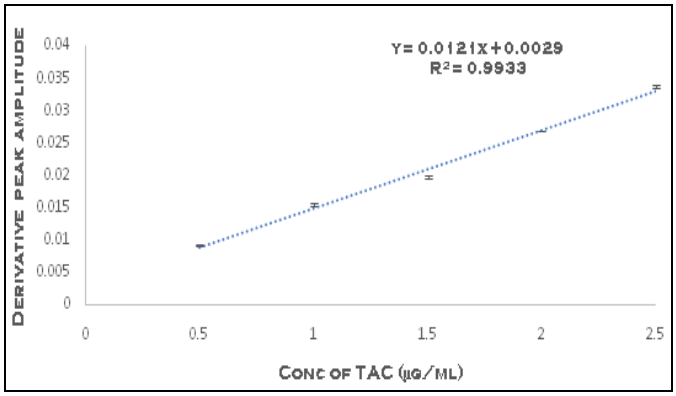

FIG. 3: CALIBRATION CURVE OF TAC AT 263 nm

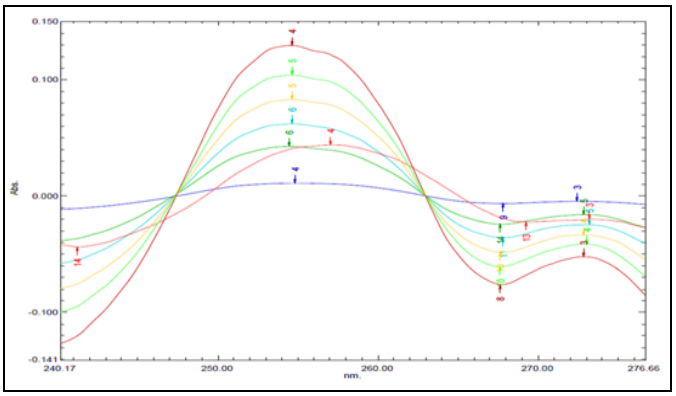

FIG. 4: OVERLAY SPECTRA OF TAC (0.5-2.5 µg/ml)

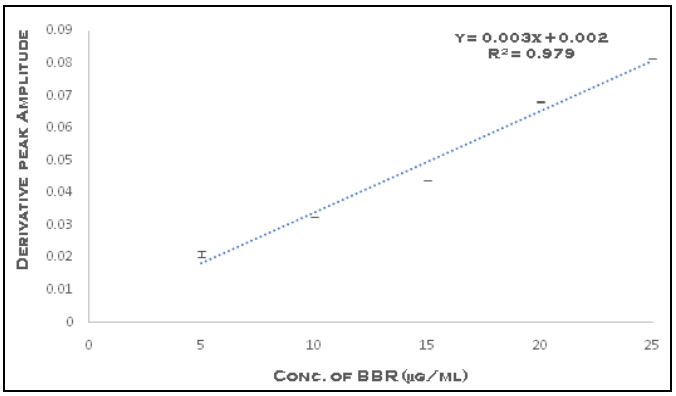

FIG. 5: CALIBRATION CURVE OF BBR AT 369 nm

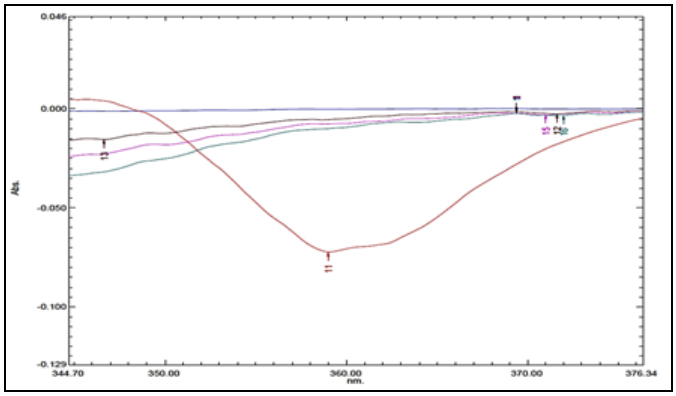

FIG. 6: OVERLAY SPECTRA OF BBR (5-30 µg/ml)

Accuracy:

TABLE 2: ACCURACY RESULTS

TAC				BBR		
263nm				369nm		
Recovery Level (%)	Initial amount of formulation (µg/mL)	Standard added (µg/mL)	% Recovery Mean ± SD*	Initial amount of formulation (µg/mL)	Standard added (µg/mL)	% Recovery Mean ± SD*
50	5	2.5	97.78±1.47	5	5	98.21±2.09
100		5.0	100.31±0.52		10	98.74±1.64
150		7.5	99.74±1.16		15	101.21±0.39

Summary of Validation Parameters of Derivative (Zero Crossing) Method:

TABLE 3: VALIDATION PARAMETERS

Parameters	TAC	BBR
Detection wavelengths (nm)	263	369
Linearity range (µg/mL)	0.5-2.5	5-25
Correlation coefficient	0.9933	0.9796
Regression Equation	y= 0.0121x + 0.0029	y=0.0031x+0.0025
Precision, %RSD		
Intra-day (n=3)	101.85, 0.74% RSD	100.72, 0.21% RSD
Inter-day (n=3)	98.71, 1.73% RSD	103.56, 0.49% RSD
Repeatability of measurement (n=6)	99.64, 0.54% RSD	101.32, 0.67% RSD
Accuracy (% Recovery, n=3)		
50 %	97.78±1.47	98.21±2.09
100%	100.31±0.52	98.74±1.64
150%	99.74±1.16	101.21±0.39
Specificity	No interferences	
LOD (µg/mL)	0.25	1.36
LOQ (µg/mL)	0.75	4.13

CONCLUSION: The present results provide clear evidence that the proposed method can be successfully used for simultaneous determination of a mixture of Tacrolimus and Berberine hydrochloride in any pharmaceutical formulation developed in the near future.

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