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Exploring Preformulation Characteristics and UV Method Development of Clobetasol Propionate for Enhanced Formulation Design

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KEYWORDS

clobetasol propionate, preformulation profiling, UV method development, formulation design, solubility, spectrophotometry.

ABSTRACT:

In order to enhance formulation design, the current work aimed to perform preformulation studies and developed a UV technique for clobetasol propionate analysis. Clobetasol propionate is a corticosteroid that is used to treat a number of inflammatory skin conditions. A thorough preformulation profiling was performed to evaluate the drug's physicochemical properties, such as solubility, partition coefficient, pH-solubility profile, and stability. The findings provided useful insights into the drug's behaviour and aided in the selection of appropriate formulation options.

Additionally, a UV spectrophotometric method for quantifying clobetasol propionate was devised. The method's wavelength selection, linearity, accuracy, precision, were all optimized. In terms of roubestness, LOD, LOQ and repeatability, the validated UV technique produced satisfactory results.

The combination of preformulation profiling and UV method development gave a thorough grasp of the drug's properties as well as a dependable analytical instrument for its measurement. The gathered data can be used to build clobetasol propionate formulations, allowing for the development of optimised pharmaceutical products with improved efficacy and stability.

1. INTRODUCTION

Clobetasol propionate (CP) is a di-halogenated derivative of prednisolone that belongs to BCS class II. It is the most commonly utilised therapy option for psoriasis. CP inhibits the transcription of pro-inflammatory cytokines that are involved in skin disease. Interferon gamma (IFN-), tumour necrosis factor-alpha (TNF-), and interleukins are examples of these. CP also increases the expression of lymphocyte genes for pro-inflammatory cytokines such IL-10 and growth factor-. CP plays an important function in rebalancing the T cells, Th1 to Th2 helper ratio in psoriatic lesions by modulating the cytokine production pathway. This medication also suppresses T helper cell growth. Furthermore, anti-mitotic activity promotes vasoconstriction and cytotoxicity, as well as anti-inflammatory activity.[1-2]

2. MATERIAL AND METHODS

Clobetasol Propionate was obtained as a gift sample from Orison Pharma International, Kala Amb, Himachal Pradesh. Methanol, ortho phosphoric acid, triethylamine and other excipients were obtained from V.K chemicals Ambala. Clobetasol 17-Propionate (CP) was obtained from SigmaAldrich, USA. Ethanol was procured from S D Fine- Chem Ltd, Mumbai, India. Formulation (ointment) collected from market with drug equivalent to 0.05% w/wof CP. All the other reagents and chemicals used were ofanalytical grade.

2.1 Preformulation studies

In order to establish a suitable dosage form for a drug, it was a pre-requisite to carryout physico-chemical properties of drug and other related properties too. Prior analysis is equally important in addition to drug development because the incompatibility between drug and excipients in the initial stages will further effects in development and retards the quality of dosage form and

Journal of Chemical Health Risks
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its therapeutic efficiency.

1) Organoleptic properties: By visual inspection, the API's organoleptic qualities—such as colour, smell, and taste—were evaluated.

2) Drug identification: The sample (drug) was analyzed by KBr pellet method using a hydraulic press. The compressed pellet was scanned at a range of wave number of 4000-400 cm^{-1} in FTIR (Bruker alpha) instrument to get the vibrational frequency of drug. The obtained FTIR spectrum sample was compared with standard spectrum of pure drug[3].

3) Melting point determination: The phase changes occur at a melting point in drug and it was identified as the temperature at which the equilibrium changes occur from solid to liquid. Melting point of the Clobetasol propionate was estimated by using Thiel's tube method. The melting range was recorded from initiation of melting point to complete phase transformation of sample[4].

4) DSC analysis: A Differential Scanning Calorimeter was used to record the DSC thermograms of API (Shimadzu, Model no: DSC-60). Samples were accurately weighted in aluminium pans, sealed and scanned from 27 to 250 °C under air atmosphere with a rate of 10 °C min-1. Al2O3 was used as reference[5].

5) Partition coefficient: Partition Coefficient Partition coefficient (oil phase/ aqueous phase) is an estimate of drug hydrophobicity and it is a marker to predict the ability of drug to cross biological membrane. Log P value greater than1 categorized as lipophillic, whereas less than 1 was an indicative for hydrophilic drug[6].

$$P_{o/w} = (C_{oil}/C_{aq})\ \text{equilibrium} \text{ ------- } 1$$

It could be approximated by measuring the distribution coefficient of CP in n- octanol/water. Clobetasol Propionate (10 mg) was added to 10 mL each of n-octanol and distilled water in separating funnel. The mixture was stirred isothermally in a circular motion (so as to prevent emulsion formation) for 30 min until drug attains equilibrium and kept aside for 1 h. The two phases were segregated and decanted separately. Analyze the drug concentration in organic phase and aqueous phase by measuring absorbance at **λ** max using UV.

6) Solubility studies: The solubility of clobetasol propionate was studied in various aqueous and non-aqueous solvents. Excess amounts of drug was added to different solvents (10 ml each) and agitated for 24 h using rotary shaker at room temperature. The solution was screened through filter paper (whatman filter paper No. 44) to obtain clear solutions, and the concentration of drug was estimated UV method[7].

2.2 Development of Method

2.2.1 Instrument name

(Variance Carry 5000, India) Double beam UV Visible Spectrophotometer

2.2.2 Preparation of Stock Solution

For the preparation of stock solution 10 mg of Clobetasol Propionate was added to 100 ml of ethanol. from this stock solution different dilutions of various concentration ranging from 2µg/ml to 40 µg/ml were made. After that the formed dilutions were checked for different parameters like accuracy, linearity, precision, LOD, robustness and LOQ[8-10].

In the method optimization process, the selection of solvent and its optimization were carried out.

2.3 Method Optimization

2.3.1 Selection of Solvent and its Optimization

According to published research, the solvent has a significant impact on the peak's appearance and quality. Solvent options for developing ultra violet methods include ethanol, methanol, acetone, etc. After testing a number of solvents, it was discovered that methanol satisfied the quality of required peak at specified wavelength[11-13].

2.3.2 Selection of Wavelength

In order to check the wavelength at which the medication has the highest absorption, a 100 g/ml solution was prepared by combining 10 mg of the medication with 100 ml of ethanol. This solution was analyzed using a UV-Visible spectrophotometer in the range of 400-200 nm, with methanol as a blank sample. The resulting spectrum, shown in Figure 2, displayed a characteristic peak at 239 nm, indicating the maximum absorption of Clobetasol 17-propionate. The chosen wavelength for further investigation is 239 nm, representing the point of highest absorption[14-18].

2.4 Validation of Method

The developed method was validated according to the Q2 (R1) recommendations of International Conference on Harmonisation (ICH) guidelines[19-20].

a) **Linearity**

This method's linearity was tested at doses ranging from 2 to 40 µg/ml. Table 1 presented data showing a linear relationship between CP's absorbance and concentration, as illustrated in Figure 3, 4 and 5. The concentrations analyzed adhered to Beer's Lambert law[21].

b) Precision

In order to ensure the accuracy of the developed UV method repeatability within a single day and its intermediate precision across different days was conducted[22].

c) Repeatability

Analysing CP at a concentration of 4 g/ml three times per day allowed for the assessment of repeatability (within the same day). The% RSD was derived from the acquired absorbance in order to evaluate the intra-day variation[23].

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4s), 156-162 | ISSN:2251- 6727

3. RESULTS AND DISCUSSION

3.1 Organoleptic Properties:

Clobetasol Propionate (CP) was found to be white to off white in colour,odourless and solid crystalline powder.

3.2 Drug Identification:

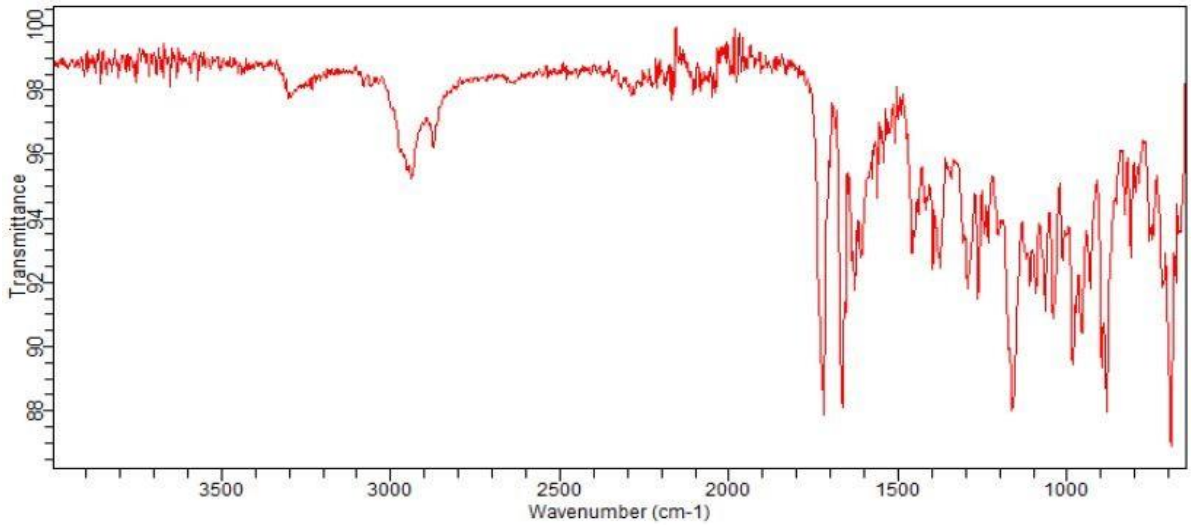

Fig1: FTIR of Clobetasol propinate

Figures 1 represent the FTIR spectra of clobetasol and physical mixtures. The IR spectrum of clobetasol exhibits characteristic absorption bands corresponding to various molecular vibrations, such as -OH stretching at 3305 cm-1, stretching of alcohol at 2952 cm-1, C=O stretching of alcohol at 1733 cm-1, C-F stretching at 1658 cm-1, and C=C stretching at 1457 cm-1.

3.3 Melting point determination:

Melting point of Clobetasol propionate was observed in the range between 195°C- 196.5ºC (literature standard value 195.5°C -197ºC).

3.4 DSC analysis

The DSC analysis of clobetasol was performed. The endothermic peak of clobetasol was obtained at 195.12˚ C which suggested that the drug is in its pure form.

3.5 Partition coefficient

The nature of the drug can be well predicted by using the partition coefficient. This difference in the drug permeation was attributed to drugproperties like pKa, log P, and solubility. In fact, the log P value was considered as a benchmark for designing of dosage form and its compatibility to the route of administration. Any molecule with a log P value greater than 2 is likely to be retained in the stratum corneum, which was true in the case of CP too. If the log P value is smaller than or equal to 2, then it was chosen as the best fit for systemic delivery.

The Log P value of clobetasol propionate was found to be 4.34. Hence it was chosen for design for topical delivery.

3.6 Solubility studies:

The solubility studies of clobetasol were done and results are depicted in the Table 1.

S. No	Solvents	Solubility of Clobetasol Propionate
1	Distilled Water	Practically Insoluble
2	Ethanol	Sparingly Soluble
3	Acetone	Freely Soluble
4	Metahnol	Soluble

Table 1: Data representing solubility studies of Clobetasol Propionate

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4s), 156-162 | ISSN:2251- 6727

3.7 Preparation of calibration curve in methanol

In order to check the linearity, the calibration curve of the drug was plotted between absorbance (taken at X axis) and concentration in µg/ml (taken at Y axis) at a wavelength of 242 nm was done.

S. No	Concentration (µg/ml)	Absorbance-1	Absorbance-2	Absorbance-3	Mean & ± S. D
1.	0	**0**	**0**	**0**	0
2.	5	0.1694	0.1701	0.1667	0.168733±0.001795
3.	10	0.3456	0.3572	0.3402	0.347667±0.008686
4.	15	0.5532	0.5599	0.5522	0.5551±0.004187
5.	20	0.7817	0.7837	0.7709	0.778767±0.006886
6.	25	0.9852	0.9902	0.9823	0.9859±0.003996
7.	30	1.193	1.199	1.109	1.167±0.050319

Table 2: Absorbance of clobetasol propionate at different concentrations

S. No	Parameter	Curve
1	Absorption maximum (nm)	239
2	Concentration (µg/ml)	5-30
3	(R^2) value	0.999
4	Regression equation	y = 0.0415x - 0.0552
6	Slope of the curve	0.0415
5	Intercept	0.0552

Table 3: Statistical parameters of calibration curve

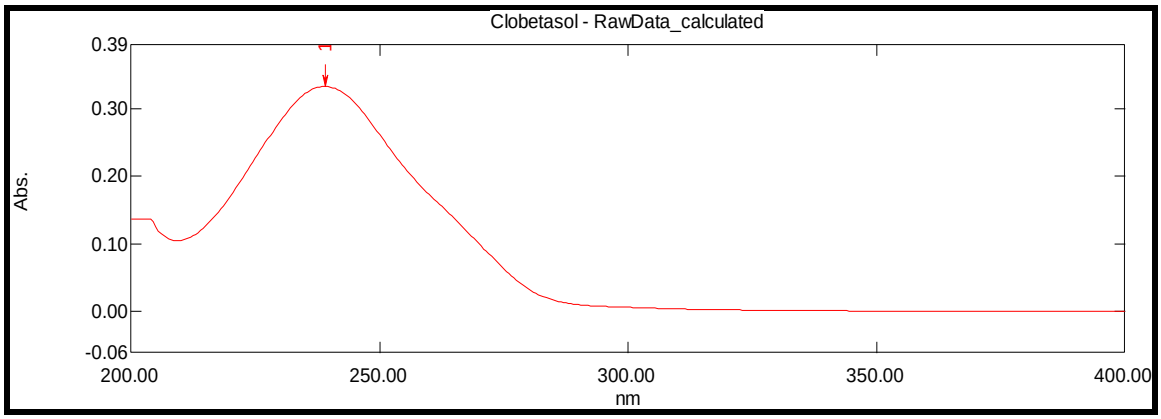

Figure 2: Absorption Maxima of clobetasol propionate in methanol

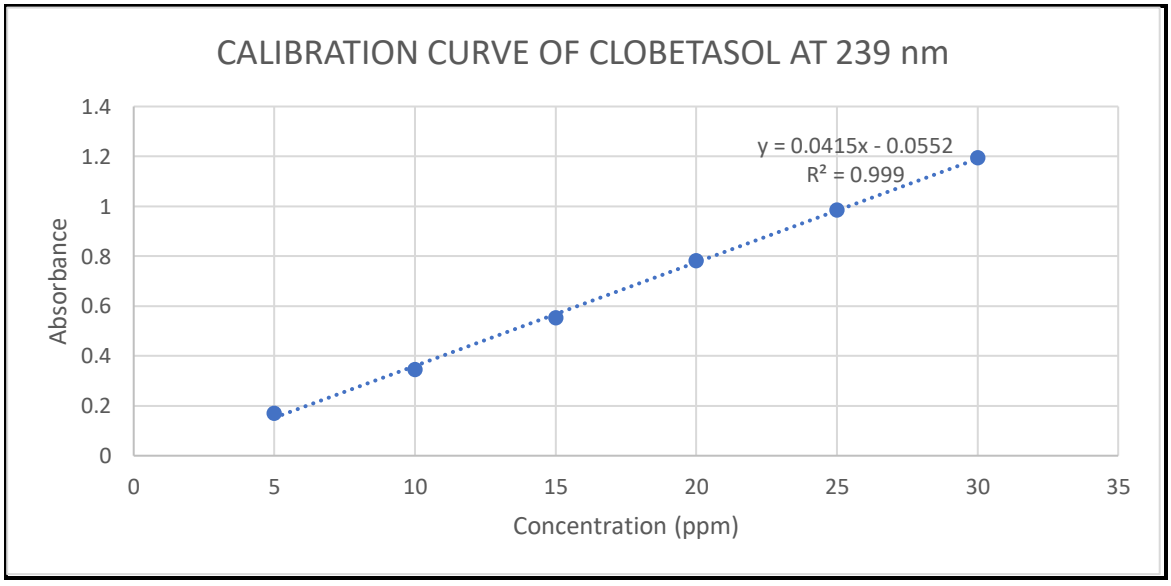

Figure 3: Calibration curve of clobetasol propionate in methanol

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4s), 156-162 | ISSN:2251- 6727

4. VALIDATION PARAMETERS

a) Linearity

The absorption maximum of CP was obtained at 239 nm which complies with λ max of official compendia (IP). The positive was observed between correlation absorbance and concentration and the regression coefficient was found to be 0.997.

b) Accuracy

The accuracy data ids depicted in the table 4.

Excess of drug added (%)	The theoretical concentration of spiked sample (µg/ml)	Conc of sample (µg/ml)	Concentration of spiked sample ± SD (µg/ml) (n=3)	Recovery ± SD (%)	%RSD
50	15	10	13.90±0.050	98.93±0.055	0.058
100	20	10	18.48±0.040	98.67±0.040	0.068
150	25	10	26.57±0.035	100.76±0.49	0.083

Table 4: Accuracy analysis of the UV method

c) Precision

Precision was determined by taking 15 ppm as test concentration. Two types of precision was done. First one i.e. system precision by making one stock concentration of 15 ppm and six times absorbance was noted done. And the second type is interday precision performed by two analyst on different days with test concentration 15 ppm. The RSD was found within limits. The precision data is shown in table Table 5.

PRECISION (15PPM)				
	SYSTEM PRECISION		INTERDAY PRECISION	
	Absorbance		Analyst 1	Analyst 2
1	0.5562		0.5562	0.5374
2	0.556		0.5523	0.5392
3	0.5589		0.5598	0.5283
4	0.5569		0.562	0.5493
5	0.559		0.5534	0.5372
6	0.5578		0.5627	0.5382
AVERAGE	0.557467		0.557733	0.538267
SD	0.001311		0.004423	0.006691
RSD	0.002352		0.007929	0.01243

Table 5: Precision recovery data of Clobetasol UV method

d) Robustness

Robustness of sample was observed by varying the wavelength ±2 nm and RSD was found within limits (Table 6).

S. No.	Conc (µg/mL)	Wavelength	
		237nm	241nm
1	15	0.542	0.5362
2	15	0.5483	0.5372
3	15	0.5444	0.5384
Mean		0.5483	0.5483
STD		0.0031	0.0011
%RSD		0.56597	0.2009

Table 6: Robustness data of Clobetasol Propinate developed U.V method

e) Validation and recovery study of marketed formulation

The validation parameter are depicted in Table 7 and recovery studies of marketed formulation is shown in Table 8.

Validation parameters	Data (Mean ± SD)
λ_{max} (nm)	239
Range (µg/ml)	2-40 µg/ml
Correlation coefficient	0.997.
Intercept	0.0162±0.00882
Slope	0.03533±0.0016
Precision (%RSD)	0.1325
Accuracy	99.93-101.84%
LOQ (µg/ml)	0.332942 µg/mL
LOD (µg/ml)	1.008916 µg/mL
Precision (%RSD)	0.007929
Robustness (%RSD)	0.2009

Table 7: Validation parameter of U.V method of Clobetasol propionate

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4s), 156-162 | ISSN:2251- 6727

Concentration level	Sample no.	Drug	Formulation	Amount added(µg/ml)	Abs.	Amount recovered	%recovery	Mean % recovered ±SD(N=3)	%RSD
50%	1 2 3		10ml of 20µg/ml	15µg/ml	0.5588 0.5746 0.5943	13.70 13.74 13.81	97.87 97.97 97.65	97.56±0.25	0.24
100%	1 2	10(µg/ml)	10 ml of 30 µg/ml	20µg/ml	0.6204 0.6802	18.91 18.70	98.57 98.56	98.04±0.01	0.02

Table 8: Recovery studies of marketed formulation

The method deployed was validated and parameters like repeatability and linearity were determined. The replications were done to predict the pure error and residual were expressed in terms of RSD, which is less than 2%.

The solubility of CP was improved when ethanol was used. The maximum absorbance of the drug, as indicated in Figure 2, was measured at a wavelength of 239 nm. The linearity of CP within the concentration range of 2 g/ml to 40 g/ml was confirmed by Table 1, with a high coefficient of correlation of 0.9999. The regression analysis in Figure 3,4 and 5 yielded the equation y = 0.0353x + 0.0162. The estimated limits of detection (LOD) and quantitation (LOQ), calculated at a factor of k = 3.3 and k = 10 respectively, were found to be 0.84 g/ml and 2.55 g/ml. Precision measurements, conducted for intra-day and inter-day analyses as presented in Table 5, exhibited a % RSD (relative standard deviation) of less than 2.0, indicating excellent repeatability of the method. The accuracy of the method was evidenced by the percent recovery values exceeding 100% in Table 4. Furthermore, commercially available ointment formulations demonstrated a CP content estimation of 98-99%.

Conclusion

The proposed approach for consistently estimating CP was found to be straightforward, accurate, exact, sensitive, inexpensive, repeatable, and quick. Without the involvement of excipients, the proposed approach is specifically designed for assessment of commercial formulations like ointments.

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