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Method Validation of Newly Synthesized Prodrugs of Aceclofenac by UV-Spectroscopy

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ABSTRACT

Prodrug designing is an important and fruitful area of drug research. Two ester-based prodrugs (**1,2**) of aceclofenac were prepared and their analytical method developed. The present study focuses on the validation of the method developed for analysis of the prodrugs (**1,2**). ICH guidelines have been followed to validate the method. Validation included limits of detection, linearity, range, and assay method. It was found simple, precise, cost effective and less time consuming.

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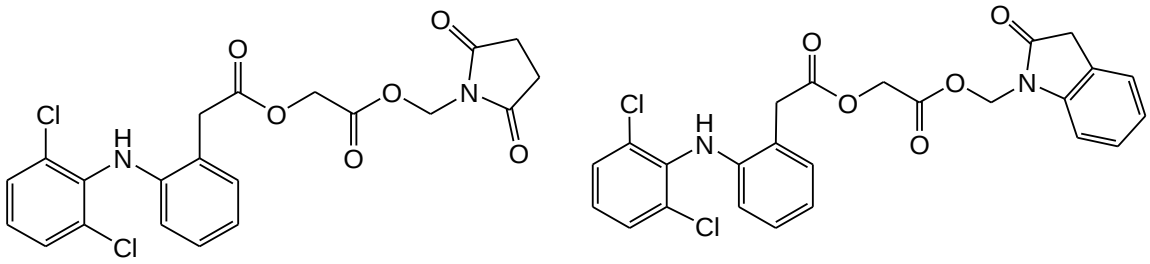
INTRODUCTION

NSAIDs (non-steroidal anti-inflammatory drugs) are of great clinical importance but their potential side effects on the stomach is are limiting factor[1,2]. NSAIDs with free carboxylic group produce gastrointestinal side effects like gastric irritation, ulceration, bleeding and perforation. Aceclofenac, an important NSAID, is used in different conditions of pain and inflammation including rheumatoid arthritis, spondylitis, and osteoarthritis[3].

A prodrug may be defined as a bio-inactive derivative of drug molecule that usually requires a chemical or enzymatic transformation within the body to release the parent drug[4]. Prodrugs may have improved pharmacokinetic, pharmacodynamic, physicochemical, and pharmacological properties over the parent drug molecule[4-7].

Analytical method development and validation play an important role in discovery, development, improvement, and manufacture of pharmaceuticals. Various analytical methodologies are employed to determine related components in different pharmaceutical formulations. There is a huge need for development and validation of new analytical methods for quality evaluation and improvement of new drugs. Once an analytical method is developed for its intended use, it must be validated[8]. The extent of validation evolves with the drug development phase. Usually, a limited validation is carried out to support an Investigational New Drug (IND) application and a more extensive validation for New Drug Application (NDA) and Marketing Authorization Application (MAA). Typical parameters recommended by FDA, USP, and ICH include specificity, linearity & range, precision, accuracy (recovery), solution stability, limit of detection (LOD), limit of quantification (LOQ) and robustness[8,9]**.**

In our previous papers[10,11], we have discussed the synthesis and evaluation of two new prodrugs (**1,2**) of aceclofenac including method development by UV-spectroscopy. Prodrug 1 and 2 of aceclofenac (**Fig. 1**) were synthesized using *N*-hydroxymethyl succinimide and *N*-hydroxymethyl isatin as promoities, respectively. In the present paper we report the validation of the analytical method of the prodrugs (**1,2**) by UV spectroscopy.



Prodrug **1** (Aceclofenac-*N*-hydroxymethyl succinimide) Prodrug **2** (Aceclofenac-*N*-hydroxymethyl isatin)

Fig. 1: Structure of the aceclofenac prodrug **1** and **2**.

MATERIALS AND METHODS

Reagents and Instruments

The chemicals and solvents used in the study were commercially procured from E. Merck (India) Ltd. and S. D. Fine. UV spectrophotometer model- Spectrum SP2000UV was used analysis. Whatmann filter paper number 41 was used for filtration. Bath sonicator was used for sonication of solutions. Phosphate buffer solution of pH 7.4 (PBS) was used as a dissolution medium for the assay. Different procedures followed for method development and validation were according to the ICH guidelines[12-18].

Working standard solution

Prodrug (**1,2**) (100 mg) was dissolved in methanol and phosphate buffer (pH 7.4) was added and the volume was made upto 100 mL (stock solution). 10 mL of the stock solution was further diluted to 100 mL with PBS to obtain a working standard solution containing 100 µg/mL.

Linearity and calibration

The aliquots of working standard solution were diluted serially with sufficient PBS to obtain the concentration range of 5–50 µg/mL. A calibration curve for aceclofenac prodrugs (1,2) was obtained by measuring the absorbance at the λ_{max} of 289 nm for prodrug **1** and 293 nm for prodrug **2**. Statistical parameters like the slope, intercept, coefficient of correlation, standard deviation, Relative standard deviation, and error were determined **(Table 1)**.

Table 1: Parameters for prodrugs 1 and 2

Parameter	Prodrug 1	Prodrug 2
Absorption maxima	289 nm	293 nm
Beer's law limit	0-35 µg/mL	0-35 µg/mL
Coefficient of correlation	0.9987	0.9513
Regression equation	Y=0.0072x+0.0184	Y=0.004x+0.003
Y intercept	0.02523	0.03865
Slope	0.0250	0.0315

Assay procedure

Accurately weighed 2 mg of the prodrug (**1,2**) was transferred to 100mL volumetric flask and made the volume to mark with PBS. This mixture was sonicated in bath sonicator for 45 minutes and filtered through Whatmann filter paper No. 41. Transferred 5 mL of the

filtrate into a 50 mL volumetric flask and made the volume to mark with PBS. Aliquots of the sample were removed and diluted to 10 mL with PBS to obtain strengths of 2, 4, 6 µg/mL, and determined for absorbance at 289 nm and 293 nm for prodrug **1** and prodrug **2**, respectively against the PBS as blank **(Table 2)**.

Table 2: Analysis results of prodrugs 1 and 2

Drug	Detection wavelength	Conc. (µg/mL)	% Purity of PBS (standard)	% Purity of prodrug
Prodrug **1**	289 nm	2	98.00	98
		4	100.78	88.08
		6	98.70	92.08
			98.5 % (mean)	92.72% (mean)
Prodrug **2**	293 nm	2	100.04	99.41
		4	99.20	99.23
		6	100.41	100.07
			99.88% (mean)	99.57% (mean)

Limit of detection: LOD was found to be 0.18 µg/mL for prodrug **1**, and 0.27 µg/mL for prodrug **2**.

RESULTS AND DISCUSSION

Once an analytical method is developed for its proposed use, it should be validated. The extent of validation involves the different drug development phases. Usually, a limited validation is carried out to support an Investigational New Drug (IND) application and a more extensive validation for New Drug Application (NDA) and Marketing Authorization Application (MAA). Typical parameters recommended by FDA, USP, and ICH include specificity, linearity & range, precision, accuracy (recovery), solution stability, limit of detection (LOD), limit of quantification (LOQ) and robustness[8,9,15-18]**.**

Two prodrugs (1,2) of aceclofenac were synthesized and their analytical method developed[10,11]. The method was validated in the present study following standard procedures. The UV scan of standard solution at 200–400 nm showed the absorption maxima of prodrug **1** at 289 nm and for prodrug **2** at 293 nm. The Beer's law was verified from the calibration curve by plotting a graph of concentration versus. absorbance[10]. Regression analysis showed very good correlation. The calibration plot revealed intercept for prodrug **1** at 0.02523 and for prodrug **2** at 0.03865 which is clear by the regression analysis equation Y = mX + C. (Where Y is absorbance, m is the slope and X is the concentration of aceclofenac in µg/mL) as obtained by the least square method. The results obtained are depicted in **Table 1**. The results of analysis for assay are shown in **Table 2**.

CONCLUSION

The proposed method for validation includes the LOD, linearity and range and assay method for the synthesized prodrugs **1, 2** of aceclofenac. The method was found to be simple, precise, cost effective and less time consuming. It can be used for general quality control analysis of the aceclofenac prodrugs **1, 2,** if future attempts are made for formulating the product.

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