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SYNTHESIS AND ANALYTICAL METHOD DEVELOPMENT OF A NEW PRODRUG OF ACECLOFENAC

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ABSTRACT: In the present work, a prodrug (2) of aceclofenac was synthesized using N-hydroxymethylisatin (1) as promoiety. Its structure was established on the basis of modern analytical techniques. An analytical, rapid, cost-effective and accurate method using UV-spectroscopy has been developed for the synthesized prodrug. The results and calibration curves obtained with different solvents for the prodrug revealed that the method developed was precise and accurate.

INTRODUCTION: The main aim of medicinal chemistry research in the recent times has been to develop drugs with enhanced efficacy, reduced toxicity and side effects. The non-steroidal anti-inflammatory drugs (NSAIDs) are of immense clinical significance but their potentially deleterious effects on the stomach are eminent [1, 2]. NSAIDs with free carboxylic group produce gastrointestinal side effects like gastric irritation, ulceration, bleeding and perforation. Aceclofenac, a NSAIDs, is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis [3]. A prodrug is defined as a biologically inactive derivative of a parent drug molecule that usually requires a chemical or enzymatic transformation within the body to release the active drug, and possess improved delivery properties over the parent molecule [4-6].

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Analytical method development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. Various analytical methodologies are employed for the determination of related components in pharmaceuticals. There is a great need for development of new analytical methods for quality evaluation of new emerging drugs. In continuation of our work on prodrugs [7-9], we report in this paper the synthesis of a prodrug of aceclofenac using *N*-hydroxymethylisatin as a promoiety and analytical method development for the same by UV spectroscopy.

MATERIALS AND METHODS:

All other chemicals and solvents used were commercially procured from various chemical units like E. Merck (India) Ltd. and S.D. Fine. Melting points were taken in open capillary tubes and are uncorrected. ^{1}H NMR spectrum was recorded on Bruker spectropsin DPX-300MHz with tetramethylsilane as internal standard in solvent $CDCl_3$. Mass spectrum was recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Spectral data are consistent with

the assigned structures. Microanalysis of the compounds was done on Perkin-Elmer model 240 analyzer and the values were found within ±0.4% of the theoretical values. Whatmann paper no. 1 was used for vacuum filtration. The progress of the reaction was monitored on TLC, which was performed on silica gel (Merck No. 5554) in the solvent systems; Benzene: Acetone (9:1) and Toluene: Ethyl acetate: Formic acid (5:4:1). Iodine chamber and UV-lamp were used for visualization of TLC spots. Dry solvents were used throughout.

Synthesis:

Two steps involved in the synthesis of the aceclofenac prodrug.

Step 1: Synthesis of *N* – Hydroxymethylisatin (**1**)

A suspension of isatin (1 gm) in formaldehyde solution was refluxed for 5 h. The hot solution was filtered, cooled and left overnight. A solid mass separated out which was filtered, dried, and crystallized from ethyl acetate to furnish a TLC pure compound, m.p. 150-152 °C. Rf value 0.82 in Toluene: Ethyl acetate: Formic acid (5:4:1).

Step-2: Synthesis of prodrug (**2**)

Aceclofenac (4mmol; 1.417 g) and *N*-Hydroxymethylisatin (equimolar, 4mmol; 0.709 g) were reacted in dry pyridine (5 mL) in the presence of phosphorus oxychloride (0.5 mL) maintaining the temperature below 5 °C. After completion of the reaction the contents were poured into ice cold water in small portions while stirring. A solid mass separated out which was filtered, washed with water, dried and crystallized from methanol to give pure compound, m.p. 135 °C. Rf value 0.71 (benzene:acetone, 9:1).

Procedure for method development: [9-11]

Prodrug (**2**) (100 mg) was dissolved in methanol and phosphate buffer (pH 7.4) was added and the volume was made upto 100 mL. This was stock solution with concentration of 1mg/mL. It was further diluted with various solvents and the absorbance was observed in order to prepare calibration curves. Different volumes were drawn from the stock solution (5,10,15,25 and 35 mL) and diluted with four different solvents (methanol, ethanol, dimethylsulphoxide and acetone) to make up the volume to 100mL. The prepared solutions were scanned in a UV-spectrophotometer. From the different absorbance values obtained the wavelength selected was 293nm for the synthesized compound (**2**).

TABLE 1: ABSORBANCE DATA OF THE PRODRUG (2) IN DIFFERENT SOLVENTS.

S. No.	Conc. (µg/mL)	Absorbance			
		Methanol	Ethanol	DMSO	Acetone
1.	5	0.107	0.098	0.024	0.107
2.	10	0.112	0.107	0.057	0.119
3.	15	0.145	0.115	0.082	0.127
4.	25	0.168	0.135	0.125	0.154
5.	35	0.207	0.147	0.148	0.178

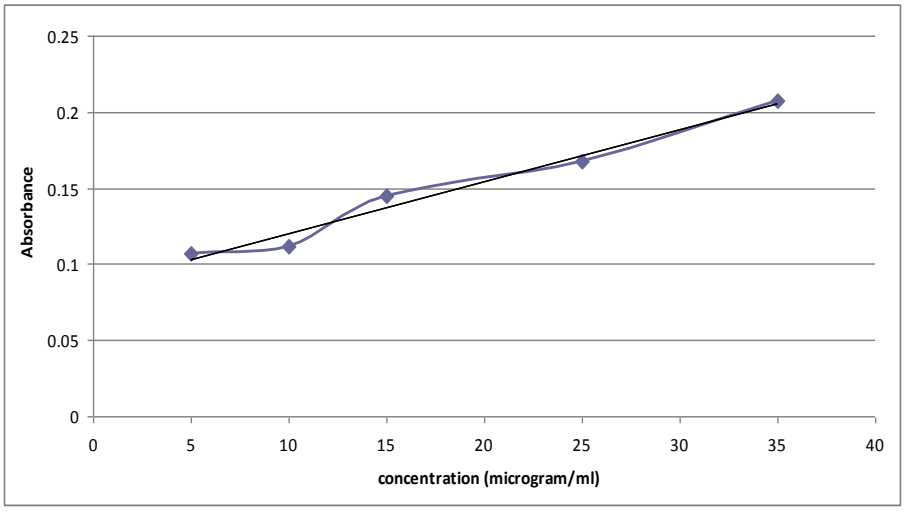


FIG.1: CALIBRATION CURVE 1: IN METHANOL

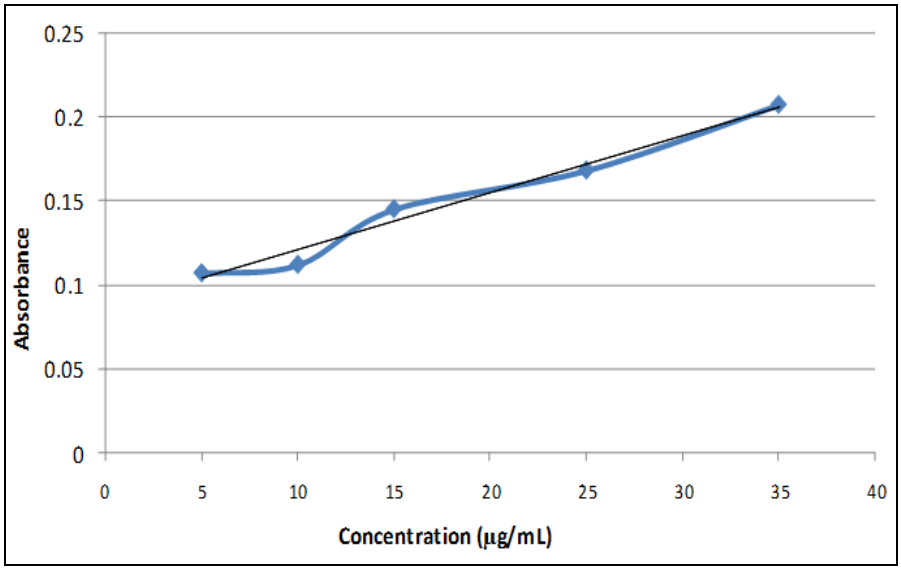


FIG.2: CALIBRATION CURVE 2: IN ETHANOL

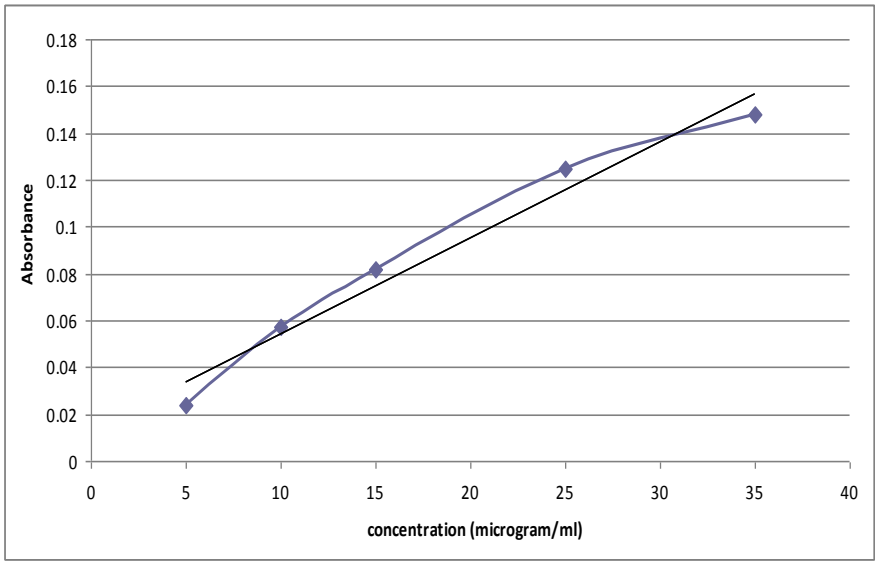


FIG.3: CALIBRATION CURVE 3: IN DMSO

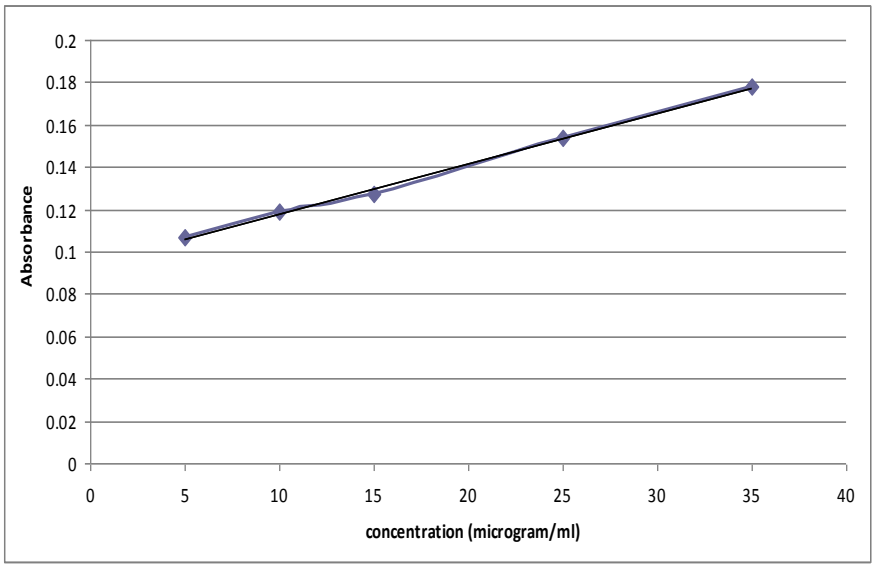


FIG.4: CALIBRATION CURVE 4: IN ACETONE

TABLE 2: CORRELATION COEFFICIENT OF THE PRODRUG (2) IN DIFFERENT SOLVENTS.

S. No.	Reagents	Value of R^2
1.	Methanol	0.8854
2.	Ethanol	0.9877
3.	DMSO	1.324
4.	Acetone	0.9997

RESULTS AND DISCUSSION:

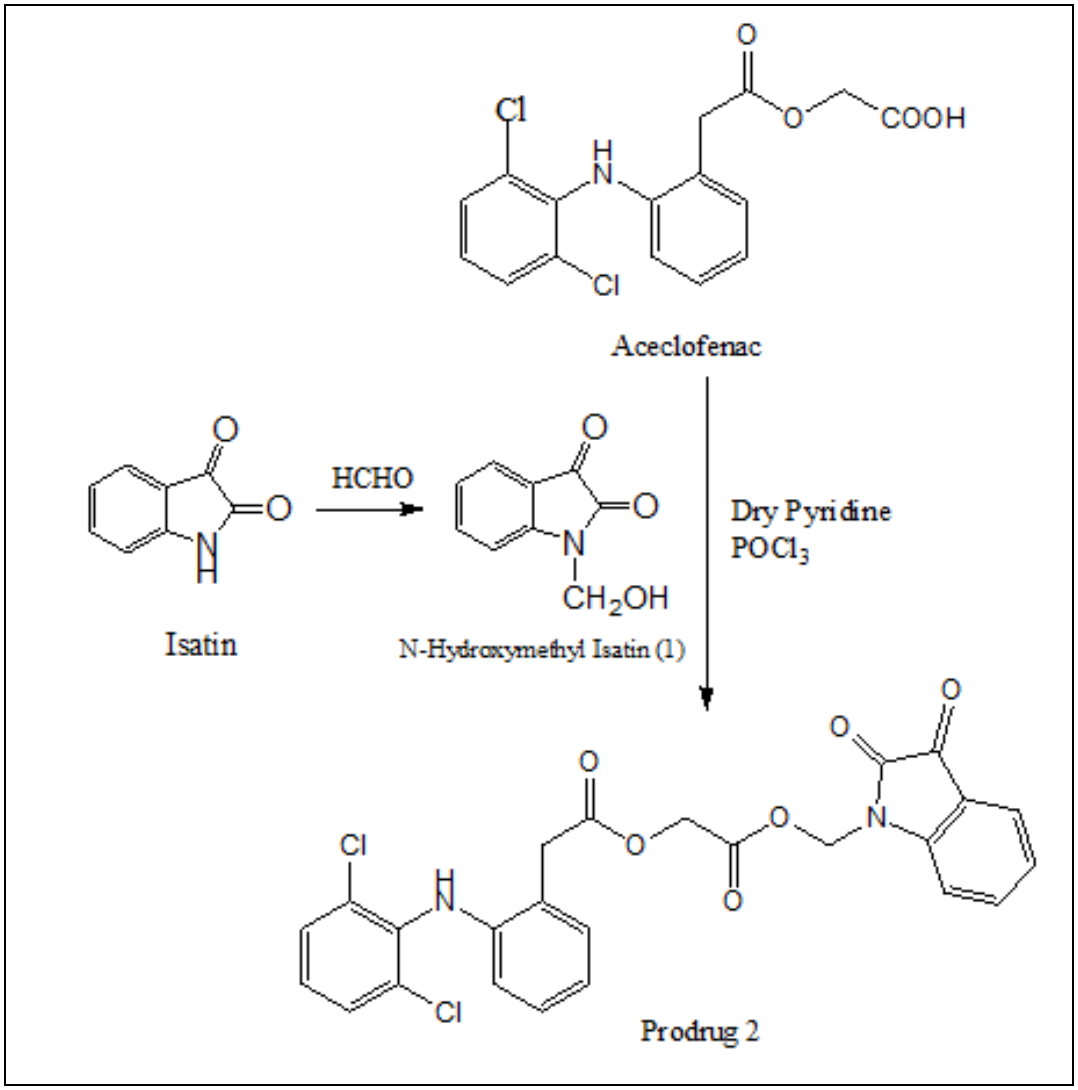
Synthesis:

Two steps involved in the synthesis of the aceclofenac prodrug 2 (**Scheme1**).

Step 1: Synthesis of *N*-Hydroxymethylisatin (**1**) Isatin was reacted with formaldehyde to obtain compound (**1**). **IR** spectral data (KBr/ν_{max} cm^{-1}): 1411 (O-H), 3034 (Ar C-H), 1678 (C=O, ketone). ^{1}H NMR (δ in ppm): 4.62 (s, 2H, CH_2), 7.04-7.09 (m, 1H, H_b), 7.17 (dd, 1H, H_d), 7.26-7.31 (m, 1H, H_c), 7.69 (dd, 1H, H_a), 11.04 (s, 1H, OH).

Step-2: Synthesis of prodrug (**2**) Aceclofenac was condensed with *N*-Hydroxymethylisatin (1) in dry pyridine in the presence of phosphorus oxychloride. Usual workup of the reaction mixture followed by crystallization from methanol furnished the prodrug (2).

Its structure was established on the basis of IR, ^{1}H-NMR and mass spectral data. IR spectral data (KBr/ν_{max} cm^{-1}): 3072 (Ar C-H), 1666 (C=O, ketone), 1515 (N-H, aromatic amine), 1341 (C-N, aromatic amine), 1297 (C-O, ester), 1091 (C-Cl). ^{1}H-NMR (δ in ppm): 3.88 (s, 2H, CH_2CO), 4.22 (s, 2H, CH_2N), 4.77 (s, 2H, OCH_2CO), 5.80 (s, 1H, NH), 7.05-7.18 (m, 3H, ring A), 6.87-6.93 (m, 4H, ring B), 7.45-7.59 (m, 4H, ring C). Mass (m/z) 512 (M^+), 513 (M+1), 514 (M+2). Elemental anal, calc, C 58.49, H 3.53, N 5.46, anal C 58.26, H 3.41, N 5.58.



SCHEME 1: SYNTHESIS PROTOCOL FOR THE PREPARATIONOF PRODRUG 2

Analytical method development process:
Prodrug (**2**) (100 mg) was dissolved in methanol and phosphate buffer (pH 7.4) was added and the volume was made upto 100 mL. This was stock solution with concentration of 1mg/mL. It was further diluted with various solvents and the absorbance was observed (scanned at 200-400nm in a UV-spectrophotometer) in order to prepare calibration curves. From the different absorbance values obtained the wavelength selected was 293nm for the synthesized compound (**2**).

After finding out the wavelength of the prodrug, the compound is further subjected to the UV-spectroscopy method development. For this, 100mg of the prodrug was dissolved in phosphate buffer (7.4pH) solution. The stock solution was prepared of concentration 1mg/mL. This stock solution was further divided into different concentrations of 5,10,15,25 and 35 mL and the solvents which were used to make up the volume upto 100mL were methanol, ethanol, dimethylsulphoxide (DMSO) and acetone.

On the basis of the results obtained and the calibration curves, the values of r square were calculated and found out to be 0.8854, 0.9877, 1.324, 0.9997 in methanol, ethanol, DMSO, acetone, respectively. It was found that the synthesized prodrug (**2**) gave the best results with ethanol and acetone, as the value of r square is very close to 1 with these solvents and almost straight line curves were obtained.

CONCLUSION: In this study, a new prodrug (**2**) of Aceclofenac was synthesized and its structure was established by IR, NMR and Mass Spectral data. ICH Guidelines were followed to develop a simple, rapid, accurate and cost-effective method for the prodrug by UV-Spectroscopy. On the basis of the results obtained and the calibration curves, the value of r square was calculated. Best results were obtained in ethanol and acetone, as the value of r square is very close to 1 with these solvents and almost straight line curve was obtained. So, it could be concluded that the method developed for the newly synthesized prodrug (**2**) was simple, rapid, cost-effective and accurate method.

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