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Formulation development and pharmacokinetic evaluation of celecoxib loaded hydroxyl propyl methyl cellulose (HPMC) microparticles in *in-vivo* model

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Abstract: This investigation aimed to formulate Celecoxib-loaded microparticles for targeted delivery to the colon, employing Hydroxypropylmethylcellulose (HPMC). Celecoxib's effectiveness in managing colorectal cancer (CRC)-related pain and inflammation is compromised by its low solubility and gastrointestinal side effects. A total of seventeen formulations were synthesized through emulsion solvent evaporation (ESE) oil-in-oil technique. The physicochemical properties of the formulations were analyzed through Fourier Transform Infrared Spectroscopy, X-ray Diffraction, Scanning Electron Microscopy and Differential Scanning Calorimetry; however, rabbits served as the biological specimens in the in vivo pharmacokinetic evaluation in Rabbits plasma. Celecoxib pure drug, F1 and F2 were given to the rabbits one time dose to assess the pharmacokinetics evaluation. The average particle size for F 1 was 497.93µm and 497.93 µm for F 2. In vitro showed cumulative release rates of 88.77% for F1 and 88.85% F2 after 24 hours. FTIR analysis confirmed the compatibility of the formulation ingredients, XRD revealed a transition of Celecoxib from its crystalline to amorphous form, DSC indicated the formulations' thermal stability and SEM images showed dense, spherical microparticles. Given these factors, using HPMC micro particles to carry Celecoxib could enhance the drug delivery and released in the body and may result effective treatment with fewer side effects.

Keywords: Celecoxib, microparticles, HPMC, HPLC, pharmacokinetic study

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INTRODUCTION

A diverse malignancy, colorectal cancer, driven by genetic mutations and characterized by complex molecular pathways that contribute to tumor initiation, progression and growth. Inflammation-induced genetic and epigenetic alterations play critical roles in CRC development, with inflammatory mediators like PGE2 and signaling proteins such as tumor necrosis factor-alpha, interleukin-1 and interleukin-6 contributing to tumor progression (Hnatyszyn *et al.,* 2019). Current treatment modalities, including surgery, radiation and chemotherapy, have limitations. Surgery is effective for tumor resection but is only applicable in certain cases, while radiation and chemotherapy can cause significant side effects, including DNA damage and poor bioavailability, especially for chemotherapy drugs. CRC, the third most common cancer globally, is expected to see a 60% rise in incidence by 2030, with an estimated 3.2 million new cases by 2040 (Xi & Xu, 2021).

Celecoxib, a selective COX-2 inhibitor, has shown promise in CRC therapy due to its anti-inflammatory, anticancer and anti-angiogenic properties. It works by inhibiting COX-2 activity, reducing PGE2 levels and suppressing angiogenesis and metastasis. Although Celecoxib demonstrates potential in cancer prevention and treatment, its clinical application is limited by gastrointestinal side effects and poor solubility. To address these challenges, research has focused on developing microparticle-based drug delivery systems to improve bioavailability and reduce adverse effects. Microparticles, typically ranging from 1 to 1000 µm in size, allow for controlled release, targeted delivery and enhanced stability of the drug.

Hydroxy propyl methylcellulose (HPMC) used to develop Celecoxib- micro-scale carriers optimized for colon specific delivery. The microparticles were formed using the emulsion solvent evaporation oil-in-oil (O/O) technique. This design allowed for the systematic investigation yield, encapsulation efficiency (EE) and drug release profile and particle size. The optimized formulation was evaluated for in vitro pharmaceutical release and biocompatibility, including oral toxicity analysis in rabbits, to confirm its safety and efficacy. Hydroxy propyl methyl cellulose (HPMC) is a widely used cellulose derivative known for its biocompatibility and solubility in cold water that ensures stability and pH-sensitive release in the colon (Rowe *et al.,* 2006 Wasay *et al.,* 2022 & Hamid *et al.,* 2021). Research in colon-targeted drug delivery systems

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(CTDDS) is advancing rapidly, especially for treating localized conditions such as IBD and CRC.

These systems use pH-sensitive, time-controlled and enzyme-responsive polymers to overcome gastrointestinal barriers and ensure targeted release in the colon. Additionally, new formulations are being tested for improved bioavailability, reducing toxicity and ensuring better retention in the colon. This study represents the pioneer systematic approach through statistical design to optimize Celecoxib-loaded microparticles for colon-specific delivery. The results offer insights into the formulation of an effective, biocompatible system that improves Celecoxib's therapeutic potential for CRC while minimizing side effects, marking a significant advancement in colon targeted drug delivery system.

MATERIALS AND METHODS

Chemicals

Celecoxib and Hydroxypropylmethylcellulose (HPMC) were kindly provided by Martin Dow Marker Limited (formerly MERCK Pvt Ltd), Quetta and Hilton Pharmaceuticals, Lahore, Pakistan. Sodium hydroxide, Ethanol, n-hexane and hydrochloric acid (37%), were sourced from Evonik Roehm GmbH (Germany) and Anala R BDH Laboratory (UK). Liquid paraffin and Dichloromethane were acquired from Merck KGaA (Germany), Span-80 was obtained from Avon Chem. Ltd (UK).

The Celecoxib-loaded HPMC microparticles

The emulsification solvent evaporation oil-in-oil (O/O) method, with slight modifications, was employed to develop microparticles. Two formulations-F1 and F2-were produced through different drug-polymer proportions: 1:1 for F1 and 1:2.5 for F2, enabling distinct drug release behaviors (fast and sustained release, respectively). For the fast-release formulation (F1), 200 mg of HPMC was used, while the slow-release formulation (F2) utilized 500 mg. Both polymers were incorporated in dichloromethane and ethanol (1:1 ratio) and homogenized at 250 rpm using, magnetic stirrer.

Celecoxib 200mg was uniformly dispersed in polymer solution along with 100 mg of magnesium stearate, which acted as a droplet stabilizer (details in table 1). Separately, the external oil phase was formulated 250 ml beaker by combining Liquid paraffin 50 ml with 1% Span-80 surfactant. This external phase was then gradually incorporated the drug-polymer mixture and continuously mixed using a Eurostar IKA, WERKE tri-blade propeller at 1000 rpm over 4 to 5 hours, or until complete solvent evaporated. Once formed, the microparticles were collected by filtration through filter paper (Whatman No. 42). They were subsequently rinsed (×4-5) with 35 ml of n-hexane for paraffin removal, then at room temperature dried for 24 hours (Maqbool *et al*., 2020).

Precision and accuracy

To assess intra-day accuracy, five replicate samples were prepared for each quality control (QC) level: LQC (0.25 µg/mL), MQC (12 µg/mL) and HQC (20 µg/mL). Similarly, inter-day precision was evaluated by preparing five replicates of each QC concentration across multiple days. The reliability and developed method were proven valid evidenced by the data presented in the table, which indicates an accuracy of over 99% in biological fluids. Under the defined experimental conditions, the method demonstrated excellent repeatability and consistency across both intra-day and inter-day analyses. For intra-day precision, the relative standard deviation (RSD%) for LQC, MQC and HQC, the values were 6.04%, 2.36% and 3.78%, respectively, respectively. Inter-day precision, the RSD% values were 6.91%, 3.55% and 4.72%. These findings confirm that the method offers high precision. The accuracy ranged from 98.00% to 99.2%, further indicating consistent performance. Overall, the intra-day and inter-day precision and accuracy support the conclusion that the developed method for quantifying Celecoxib in plasma is both precise and reliable.

Robustness

To evaluate the robustness of Celecoxib separation, various chromatographic parameters were intentionally altered. The flow rate was increased by 0.3 units to assess its influence. Additionally, the solvent composition was modified by increasing the volumes of acetonitrile and water by 2 mL each and acetic acid by 0.3 mL. The impact of column age on performance was also examined by comparing results obtained using a brand-new column with those from columns aged 30, 60 and 90 days, while keeping all other conditions unchanged.

Characterization

Fourier transform infrared spectroscopy

To assess the compatibility between Celecoxib and the polymers, as well as the blend of the polymer and drug and formulation (F1 and F2) FTIR analysis carried out. A Bruker Tensor 27 German FTIR spectrophotometer utilized to scan the samples within the 4000-400 cm^{-1} range. Each sample was placed on a disc and firmly pressed, after which the sample was scanned over 16 seconds (Kashif *et al.,* 2017).

Thermal stability evaluation

DSC analysis was carried out on Celecoxib, the polymers, drug and polymer physical blend and formulation (F1 and F2) using a LABKITS-100 DSC instrument (Hong Kong). Approximately 7 ± 0.1 mg of sample was applied in an aluminum pan and thermally treated from 30-300°C under a nitrogen purge at a discharge of 20 mL/min. Indium and zinc served as calibration standards (Wasey *et al*., 2022).

X-Ray diffraction evaluation

JEOL JDX-3532 X-ray diffractometer (Japan) utilized for XRD to evaluate the crystalline structure of Celecoxib, the polymers, the drug-polymer physical mixture and

(formulation F1 and F2). Specimen were analyzed at a scan interval of 2°/min over a 5-70° range at 30 mA and 35 kV, with diffraction patterns compared to detect any shifts or changes in peak positions (Abadi *et al*., 2020).

Particle size evaluation

The yield percentage of the completely dried microparticles was determined using a 10x lens under a microscope slide setup, as described by Kashif *et al.* (2017). This was done by dividing the amount of produced obtained by the anticipated (theoretical) amount and then multiplying the result by 100 (Kashif *et al.,* 2017).

Scanning Electron Microscopy

The shape and surface morphology of the Celecoxib-loaded microparticles were analysed through JEOL JSM5910, Tokyo, Japan, scanning electron microscope. The specimen was mounted on metal bases with double-sided tape, then vacuum-dried and covered with a thin layer of gold using a sputtering process. SEM imaging was carried out at various magnifications to observe the microparticles' structure (Montejo *et al.,* 2010).

In-vitro analysis of drug release

The analysis of Celecoxib-loaded HPMC microparticles in vitro release was conducted using a chronological pH shift technique. The release was significantly higher than the medium at pH 1.2, 6.8 and 7.4 over 2, 10 and 12 hours at 37°C ± 0.5. The enhanced release was attributed to the drug's transition from crystalline to amorphous form, improving particle size and surface area (Katona *et al*., 2020). No burst release was observed, indicating uniform drug dispersion. Figure 5 compares the cumulative release of pure Celecoxib with F1 and F2 formulations at different HPMC ratios. Increasing polymer concentration raises its density, lengthening the diffusional path and reducing drug release (Sharma *et al*., 2015). In-vitro release of Celecoxib-loaded microparticles was investigated through Pharma Test, Germany Type-II dissolution apparatus at 37°C ± 0.5°C and 50 rpm, following USP guidelines. Dissolution medium at pH (1.2, 6.8 and 7.4) were applied sequentially over 2, 10 and 12 hours. A 7.5 mg Celecoxib sample was placed in a pre-soaked dialysis membrane and immersed in 450 mL of medium. Drug release occurred over 24 hours: 2 hours in pH 1.2, 10 hours in pH 6.8 and 12 hours in pH 7.4. Specimens were taken at set period, filtered and examined for absorbance using a UV spectrophotometer (IRMECO GmbH, Germany)

Pharmacokinetics Evaluation

Equipment and HPLC Conditions

HPLC technique was established for quantification of Celecoxib, utilizing an isocratic system (Syknm, Germany). The setup included a solvent delivery module (S-1122), S-3210 UV/Vis detector and Peak329 software for system control. Chromatographic separation as executed out on a C18 analytical column (Phenomenex, Luna RP-C18, 5 µm, 250 × 4.6 mm I.D.). A blend of acetonitrile, water and acetic acid in a 50:45:5 volume ratio was used as the mobile phase. Before use, the mobile phase was degassed in an ultrasonic bath (Elma, Transsonic Digitals, Germany) and purified via a 0.45 µm DURAPORE® membrane using a Millipore vacuum filtration unit (Ireland). The flow rate was kept constant at 1 mL/min, with a 20 µL sample volume introduced via the injector. Detection was performed at a wavelength of 254 nm. Additional laboratory equipment employed in the study included a Shimadzu, Kyoto, Japan digital analytical balance, a Sigma-Zentrifugen, Osterode, Germany centrifuge and a MS2 Minishaker IKA, Germany vortex mixer.

Method validation

Animals

The experimental protocol received ethical clearance from the Institutional Animal Ethical Committee (IAEC) at the Faculty of Pharmacy and Health Sciences, University of Balochistan (UOB), Quetta, Pakistan (Reference No. FoP & HS/ICE/213/20, dated November 20, 2020). A total of nine rabbits, each weighing among 2.0 and 2.5 kg, were selected for the study. The animals were aboded under standardized laboratory conditions, maintained at a temperature of 23 ± 2°C with 45% relative humidity and 12-hour light/dark cycle. Provided with a nutritionally balanced diet and had unrestricted access to clean drinking water and were appropriately tagged for identification. During the dosing and sampling procedures, the rabbits were housed in individual cages. Three groups of rabbits were formed for the study, each consisting of three animals (n = 3). After an overnight fast (Patel, 2017), the rabbits were administered an oral dose of celecoxib equivalent to 2 mg/kg body weight, as follows:

- *Group I/ Control group:* Celecoxib
- *Group II/ Test group I:* Celecoxib-loaded HPMC microparticles (F1)
- *Group III/ Test group II:* Celecoxib-loaded HPMC microparticles (F2)

Plasma extraction

From the jugular vein, blood was obtained after fur removal with a depilatory agent. Restrained in rabbits' wooden carriers and 3 mL specimens were drawn at scheduled intervals into EDTA tubes using sterile disposable syringes.

Once collected, blood specimens were centrifuged at 6,000 rpm for 30 minutes to part the plasma, preserved at -20°C until further analysis. Prior to use, the stored plasma samples were brought to room temperature to allow complete thawing before investigation. At this phase, an equal volume of acetonitrile (1:1 ratio) was added as a protein precipitant and vortexed for 4-5 minutes, centrifuged again and transferred into a hygienic Eppendorf tube and labeled. (Abadi *et al.,* 2020). Prior to

HPLC analysis, the samples were passed through 0.20 µm Sartorius Stedim Biotech GmbH, Germany Minisart syringe filters. To obtain precise results, a solvent mixture used to rinse system and column, with, for approximately 30 minutes before the analysis.

Quantification and pharmacokinetics profiling

Celecoxib levels and its pharmacokinetic profile were evaluated using PK Solver, an add-in tool for Microsoft Excel. The analysis was conducted on selected rabbit groups (n=3) to determine key pharmacokinetic indicators. Parameters, time to peak plasma concentration (Tmax), maximum plasma concentration (Cmax), area under the concentration-time curve (AUC), average residence time (MRT) and elimination half-life ($t_1/_2$), were determined using the log-linear trapezoidal technique applied to the plasma concentration-time data.

Validation of Method

ICH guidelines (Q8, 2005), thorough validation process was established to quantify Celecoxib in plasma. A calibration curve was generated from plasma samples with known Celecoxib concentrations, enabling the evaluation of key specifications as precision, linearity, specificity, accuracy and quantification (LOQ) and limits of detection (LOD).

Linearity and the standard curve

Plotting the average peak area against the Celecoxib concentration, calibration curve was generated. To assess linearity, various concentrations were tested, with peak areas plotted against their respective drug concentrations. Slope, Correlation coefficient and intercept were determined via least-squares regression.

Specificity

The Clecoxib peak, specificity, was distinct from other unwanted peaks, such as components of the solvent system and plasma proteins. Spiked plasma specimens and blank were tested for selectivity. A standard chromatogram was created to confirm other assessment in the sample matrix potentially effectively extracted from target compound. To achieve optimal resolution of the analyte, rabbit plasma samples were meticulously prepared. As a result, the method demonstrated excellent performance (Alvi *et al.,* 2021).

Accuracy and precision

Degree of consistency or repeatability of the measured values need to align for a method to be deemed reliable. It's typically articulated as the relative standard deviation (RSD %). A smaller RSD signifies higher precision, meaning the method is more consistent. Precision is generally assessed through two key parameters: repeatability and intermediate precision. Intermediate precision considers variations such as changes in testing days (inter-day) or using different instruments, while repeatability evaluates consistency under the same conditions-same day, same equipment (intra-day). To assess intra-day precision, five samples collected on the same day were analyzed repeatedly. In contrast, inter-day precision was assessed by examining five validation samples across five different days. Accuracy, which reflects how closely the results match the true values, is often referred to in terms of bias. It is determined by evaluating the average measured value to true value and relative standard error is represented (Bae *et al.,* 2007).

Limits of detection and quantification

LOD indicates, lowest amount of a substance in a sample that can be reliably identified, though not essentially measured with precision. In contrast, "limit of quantification" (LOQ) represents the smallest proportion at which analyse can be accurately and consistently quantified using a given method. LOQ was determined based on the lowest concentration from the plasma spiking curve that still met acceptable criteria for accuracy and precision-typically within a 20% margin (Krier *et al.,* 2011). Because LOD is generally one-third of the LOQ.

1. LOD = 3.3 * (Sy/S)
2. LOQ = 10(SD/S)

Analysis time

Solvents consumed, plays a crucial role in determining the total analysis time. These factors analyte, the run time may vary; however, most methods typically fall within a range of 0 to 15 minutes. The total run time was condensed to 10 minutes. It's important to note, though, that while faster run times improve efficiency, they can sometimes compromise the accuracy and reliability of the results (Alvi *et al*., 2021).

Robustness/Ruggedness

Robustness describes the method's ability to maintain performance despite minor, intentional variations in experimental conditions, highlighting its reliability under routine operating conditions. The term "ruggedness" is often used interchangeably with robustness. To evaluate this, deliberate adjustments were made to the chromatographic settings to observe their impact on Celecoxib separation. For instance, the elution was increased by 0.3 units. Additionally, the carrier solvent-comprising acetonitrile, water and acetic acid-was altered to study the influence of varying solvent concentrations. The impact of column longevity was also assessed by comparing results obtained from new columns with those from columns aged 30, 60 and 90 days and other conditions unchanged.

STATISTICAL ANALYSIS

All the results were presented as a mean with a standard deviation, ANOVA, one-way analysis of was done to assess the variance in Celecoxib in the control groups plasma and the optimized formulations SPSS v21.0 used as statistical tool. All the results were significant ($P<0.05$).

RESULTS

Characterization

FTIR analysis

The FTIR spectrum of Celecoxib shows key peaks: O-H stretch appeared broadly at 3200-3500 cm^{-1}, ester C=O at 1725 cm^{-1} and aromatic C-H stretches at 3000–3100 cm^{-1} and C=C stretch (1500-1600 cm^{-1}, benzene). The sulfonamide S=O appears at 1270 cm^{-1}, with N-H, C-N at 3300 cm^{-1} and 1200-1350 cm^{-1}. C-H bending (700-900 cm^{-1}) and C-S stretch (850 cm^{-1}) confirm sulfur linkage. The fingerprint region (600-1500 cm^{-1}) supports Celecoxib identification as depicted in fig 1.A.

HPMC displays a Broad O-H stretch at 3200-3500 cm^{-1}; C-H stretches at 2800-3000 cm^{-1}, C-O (1050-1150 cm^{-1}) and C-O-C ether peak (~1100 cm^{-1}). Methyl groups show a strong band at 2950 cm^{-1}. The fingerprint region (600-1000 cm^{-1}) confirms HPMC's structure. While both compounds share O-H and C-H stretches, Celecoxib shows sulfonamide-specific bands, whereas HPMC highlights ether and hydroxyl signals. Their distinct fingerprint regions allow clear differentiation as in fig1.B. The FTIR of the Celecoxib-HPMC formulations F1 and F2 includes features from all components. The fingerprint region confirms the presence and compatibility of all constituents as depicted in fig 1.C and D (Vijayakumar *et al*., 2016).

XRD analysis

XRD analysis of Celecoxib displays a broad halo in the low 2θ range, indicating a crystalline structure in (fig. 2A), with minimal molecular organization. This reduced order can influence the drug's solubility and absorption (Chawla *et al.,* 2003), making it a key factor in evaluating Celecoxib's solid-state characteristics in formulations. For Hydroxypropyl Methylcellulose (HPMC), XRD patterns reveal wide, diffused signals consistent with its amorphous nature shown in (fig. 2B), which supports improved solubility and sustained drug release. Minor crystalline features may arise depending on processing methods, underscoring HPMC's significance in controlled delivery systems. The reduction inpeaks intensities of Celecoxib in the formulations (figs. 2C and D), When Celecoxib is combined with HPMC, the XRD profile shows sharp peaks for Celecoxib and broad ones for HPMC. This contrast allows for monitoring changes in the drug's crystalline structure, which may enhance both solubility and bioavailability-important indicators of polymer compatibility and release behavior. In the final formulation F1 and F2, Celecoxib retains its crystalline peaks, while HPMC contribute broad amorphous signals. XRD confirms structural interactions that affect the drug's dissolution rate, physical stability and release dynamics

DSC analysis

Differential Scanning Calorimetry (DSC) analysis of Celecoxib displays a distinct endothermic peak at its melting point, confirming its crystalline form as revealed in fig. 3A. The technique also reveals thermal events such as degradation and phase transitions, offering key information on the drug's thermal behavior and stability (Chawla *et al*., 2003). For Hydroxypropyl Methylcellulose (HPMC), DSC shows a broad endothermic signal around its glass transition temperature (Tg), as shown in fig. 3B. Characteristic of its amorphous structure (Ford, 1999). The analysis also detects moisture evaporation and thermal breakdown, providing insights into HPMC's suitability for sustained-release formulations. In the Celecoxib-HPMC blend, DSC highlights changes in melting and Tg values, suggesting possible molecular interactions and modifications in crystalline structure (Chowdary & Srinivas, 2006) which are important for determining formulation stability. DSC also captures thermal degradation, offering essential data for evaluating its reliability in controlled drug delivery systems. In the combined formulation F1 and F2 containing Celecoxib, HPMC. DSC reveals a sharp melting point peak for Celecoxib, along with broad Tg signals from the polymers. Any deviations or shifts in these peaks suggest interactions between the drug and excipients (Lu *et al*., 2006) as depicted in figs.3C and D. Additionally, DSC identifies degradation or phase changes, which are vital for assessing the formulation's stability and overall performance.

Surface morphology

Scanning Electron Microscopy (SEM) images of Celecoxib display unevenly shaped crystalline particles with coarse surfaces, which can negatively influence its dissolution rate and absorption (Ha *et al*., 2015) as depicted in fig.4.A. In contrast, Hydroxypropyl Methylcellulose (HPMC) exhibits a smooth, porous morphology-an essential feature for sustained drug release-though its surface appearance can vary depending on processing techniques (Alvi *et al*., 2021). The SEM images of the HPMC reveal structure as shown in (fig. 4B), embedded within HPMC's fibrous network (Ha *et al*., 2015). In the final formulation containing Celecoxib, HPMC SEM shows Celecoxib particles retained in a smoother, rod-like matrix formed by HPMC. This structural arrangement plays a critical role in modulating drug release behavior as depicted in fig. 4.C.

In vitro drug release study

The drug release in vitro profile of Celecoxib-loaded microparticles was evaluated using Type-II, Pharma Test, Hainburg, Germany, USP dissolution equipment at 37°C ± 0.5°C and 50 rpm. Celecoxib exhibited a relatively low initial release rate of approximately 12.123%, likely due to its hydrophobic characteristics. However, the cumulative drug release from the microparticles was significantly higher than that of the pure drug. This enhanced release can be attributed to the transformation of the drug from a crystalline to an amorphous form, along with reduced particle and size improved (Katona *et al.,* 2020). Notably, none of the formulations displayed a burst release effect, suggesting uniform distribution of the drug within the

matrix. Figure 5 illustrates the comparative cumulative release profiles of pure celecoxib and two formulations (F1 and F2) incorporating different ratios of HPMC. The data showed that as the ratio of HPMC amplified, on the whole drug release rate decreased. Specifically, formulation F2, which had a lower HPMC ratio (1:1), demonstrated a higher release rate of 74.423%, whereas formulation F1, with a higher polymer content, released only 70.123% of the drug.

In vivo pharmacokinetic analysis

Method development

The initial step in establishing any chromatographic method is the development of an appropriate technique. The aim of this phase is to optimize key parameters such as sensitivity, accuracy, precision, resolution and repeatability. During the method development process, several C18 columns with 5µm particle sizes were evaluated, including the Phenomenex RP- C18 Luna 5µm columns (100 × 4.6mm, 150 × 4.6mm and 250 × 4.6mm), all from Phenomenex. Additionally, various mobile phase combinations using acetonitrile (ACN), water and acetic acid at different ratios were tested. After thorough evaluation, HPLC column RP-C18 Luna 5µm (250 × 4.6mm) was utilized. The optimal mobile phase: acetonitrile, water and acetic acid in a 55:40:5 ratios provided the best retention time, peak shape and sensitivity. The flow rate was tested within a range of 0.5-1.5 mL/min and 1 mL/min was selected as the most suitable for consistent and efficient separation. Investigation interval was maintained at 10 minutes, Celecoxib retention interval was found designated between 10.00 and 10.136 minutes as shown in fig.7A and B The pressure during the experiment was located between 110 and 130 bars and the analysis was carried out at room temperature.

Plasma calibration curve

A calibration curve for Celecoxib was created by adding known concentrations of the drug to plasma, concentrations of 0.3125, 0.625, 1.25, 2.5, 5 and 10 µg /ml were used for standard curve. Concentration of drugs placed at X-axis and peaks areas were placed at Y-axis. The average peak area was plotted versus concentration. Linear regression as depicted in fig. 6. The coefficient of determination (R^2) was 0.999, confirming a robust linear correlation within the selected concentration range

DISCUSSION

Working range

The method's linear range has been characterized by the highest and lowest analyte concentrations at which the system response is precise, linear and accurate. It was observed that the method preserved linearity within the concentration array of 0.25 to 24 µg/mL for Celecoxib in plasma. Consequently, this range was selected as the working range in rabbit plasma for Celecoxib (Alvi *et al.,* 2021).

Isolation and selectivity

A reliable reported HPLC method used for celecoxib isolation (Ziaei *et al.*, 2020). Within a total run time of less than 10.5 minutes, Celecoxib was effectively separated from rabbit plasma. The drug displayed a retention time of approximately 6.2 minutes, with no interference from endogenous plasma peaks. Under optimal chromatographic conditions, Celecoxib separation from plasma was achieved with satisfactory results. The chromatograms of Celecoxib, both in the absence and presence of plasma, are depicted in fig. 7A & B.

Limits of detection and quantification

The HPLC method was used to evaluate the sensitivity of Celecoxib extraction by determining its LOD and LOQ. The LOD for Celecoxib was found to be 0.061µg, indicating the lowest detectable concentration, though it could not be quantified. In contrast, the LOQ was analyzed to be 0.2810 µg, representing the smallest concentration at which Celecoxib could be both detected and accurately quantified.

Precision and accuracy

To assess intra-day accuracy, five replicate samples were formulated for each control concentration: LQC (0.22 µg/mL), MQC (12.10 µg/mL) and HQC (19.10µg/mL). For inter-day precision, five replicate samples for each of the LQC, MQC and HQC analyzed. The reliability and accuracy of the validated protocol are summarized in table 2. This indicates that the analysis of biological fluids achieved an accuracy exceeding 99%. Under defined experimental conditions, the method demonstrated strong repeatability, as confirmed by both inter-day and intra-day precision assessments. For intra-day testing, the relative standard deviation (RSD) values for low (LQC), medium (MQC) and high-quality (HQC) control specimens were 6.04%, 2.36% and 3.78%, respectively. Corresponding inter-day RSD values were 6.10%, 3.22% and 3.15%. These results highlight the method's high level of precision. Furthermore, the accuracy of the developed method ranged from 97.00% to 99.22%, indicating reliable and reproducible performance. Overall, the intra-day and inter-day precision and accuracy confirm that the method is both robust and dependable for the quantification of celecoxib in plasma samples. (Salunkhe *et al*., 2019).

Robustness

To evaluate how celecoxib separation is influenced, various chromatographic parameters were modified. The flow rate was increased by 0.2 units. The effect of the solvent system was examined by adjusting the volumes-adding 1 ml each to acetonitrile and water and 0.2 ml to acetic acid. Additionally, the influence of column condition was explored by comparing results obtained using a fresh column with those from an older column at intervals of 30, 60 and 90 days.

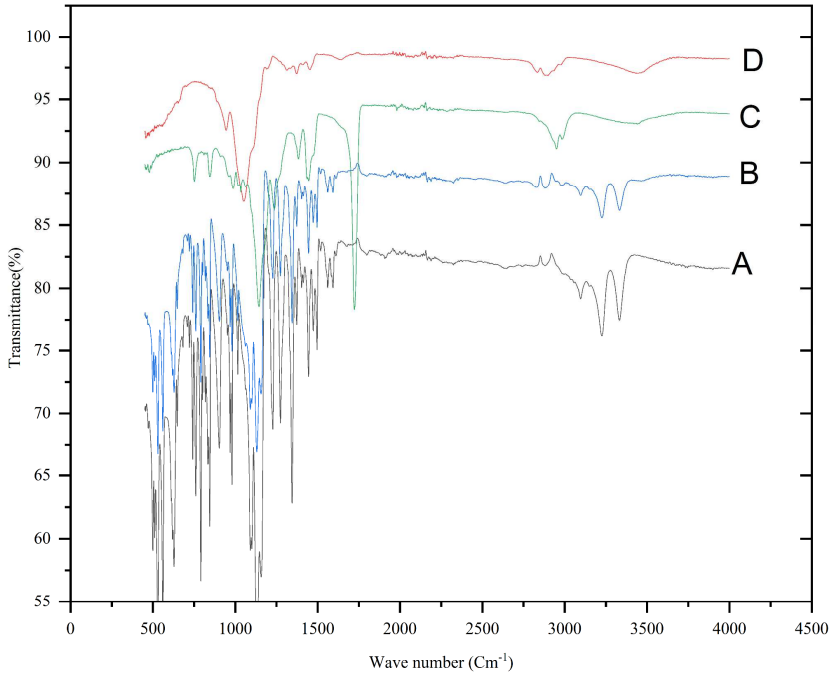


Fig. 1: Celecoxib FTIR spectra (A), HPMC (B), and microparticles F1 and F2 (C, D

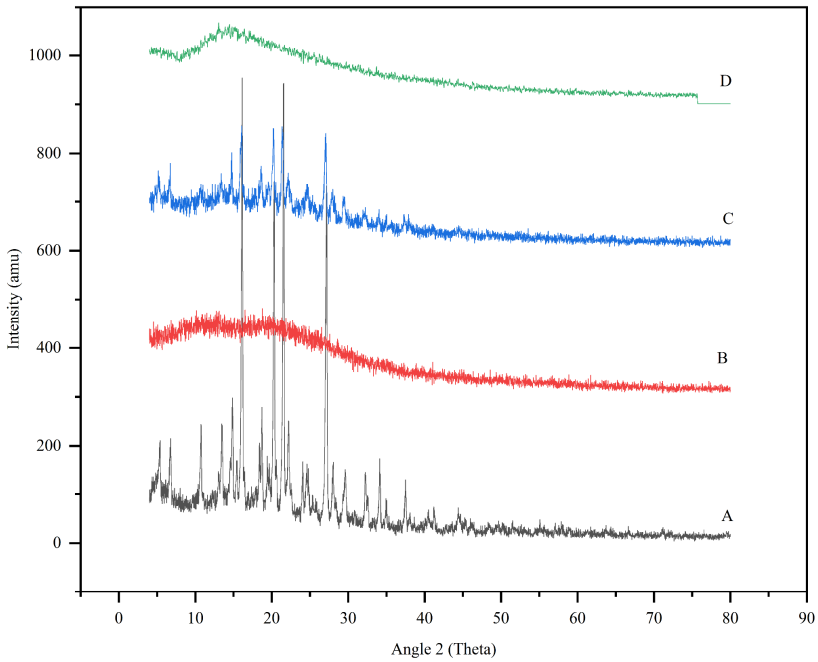


Fig. 2: Celecoxib XRD patterns (A), HPMC (B), and F1/F2 formulations (C/D).

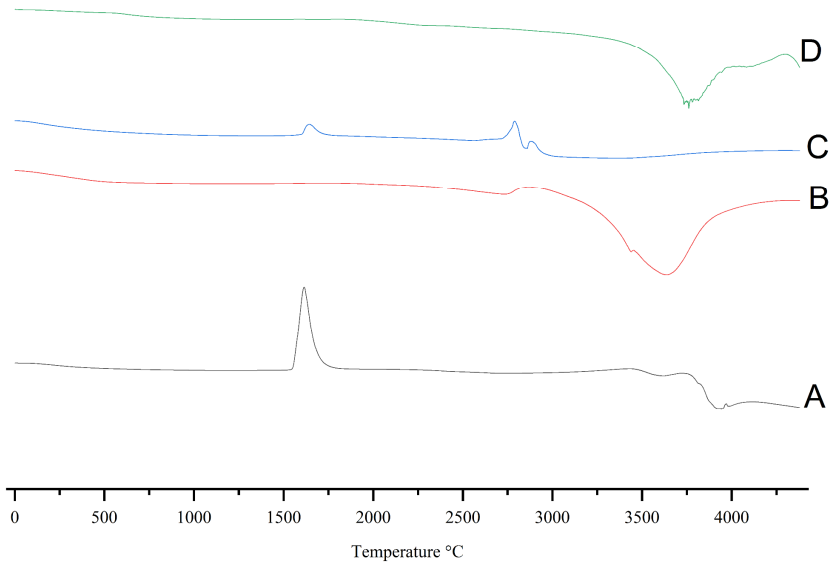
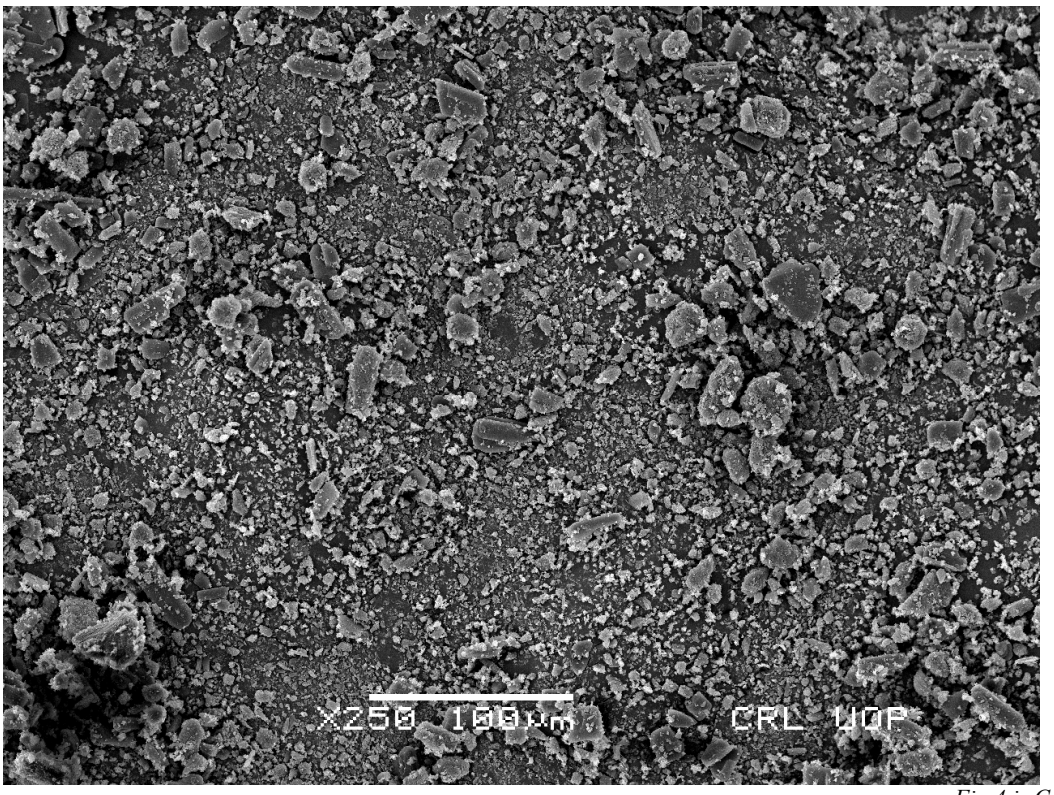


Fig. 3: Celecoxib DSC profiles (A), HPMC (B), and F1/F2 (C/D)



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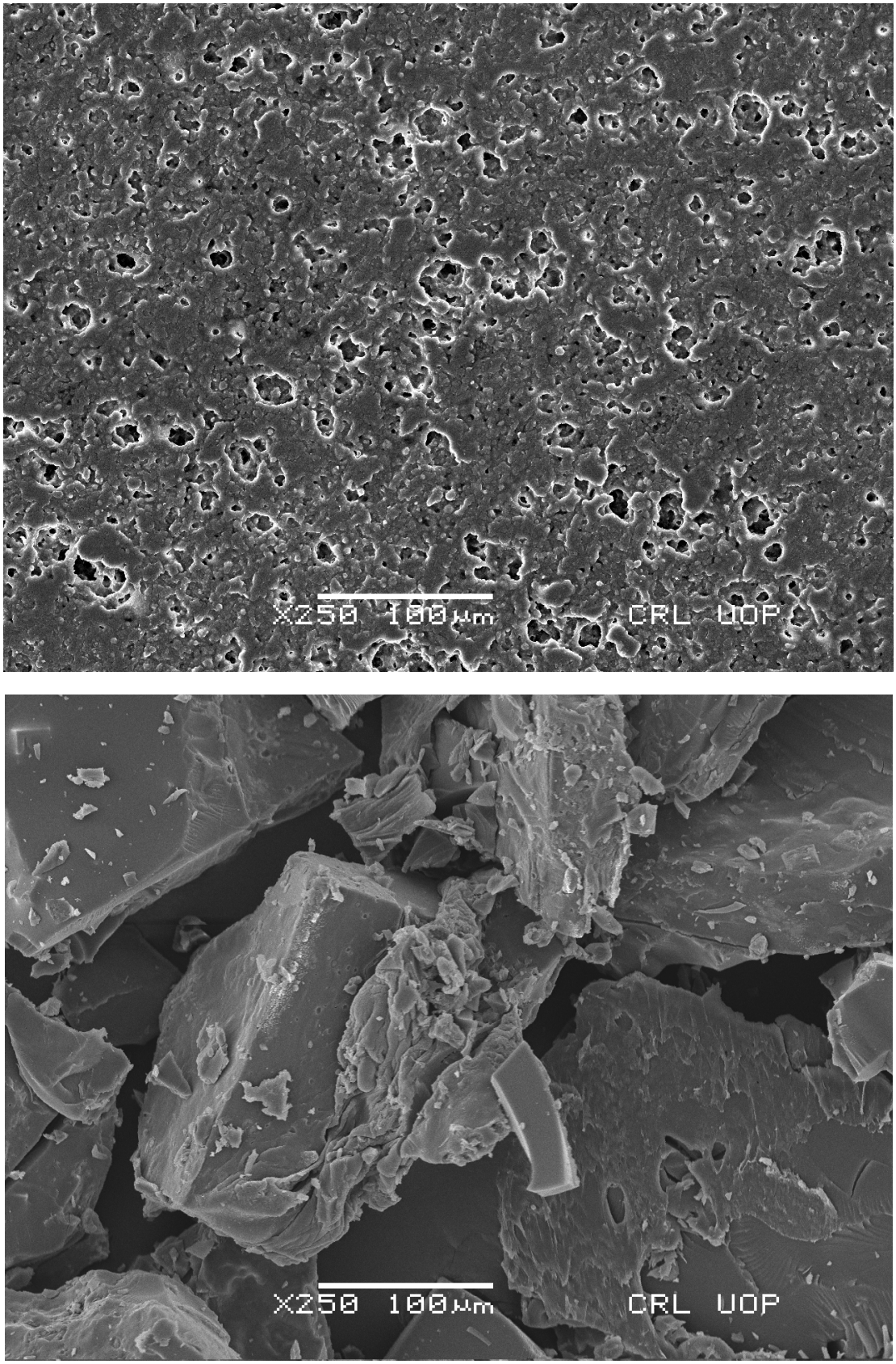

Fig. 4: SEM of Celecoxib (A), HPMC (B), and F (C).

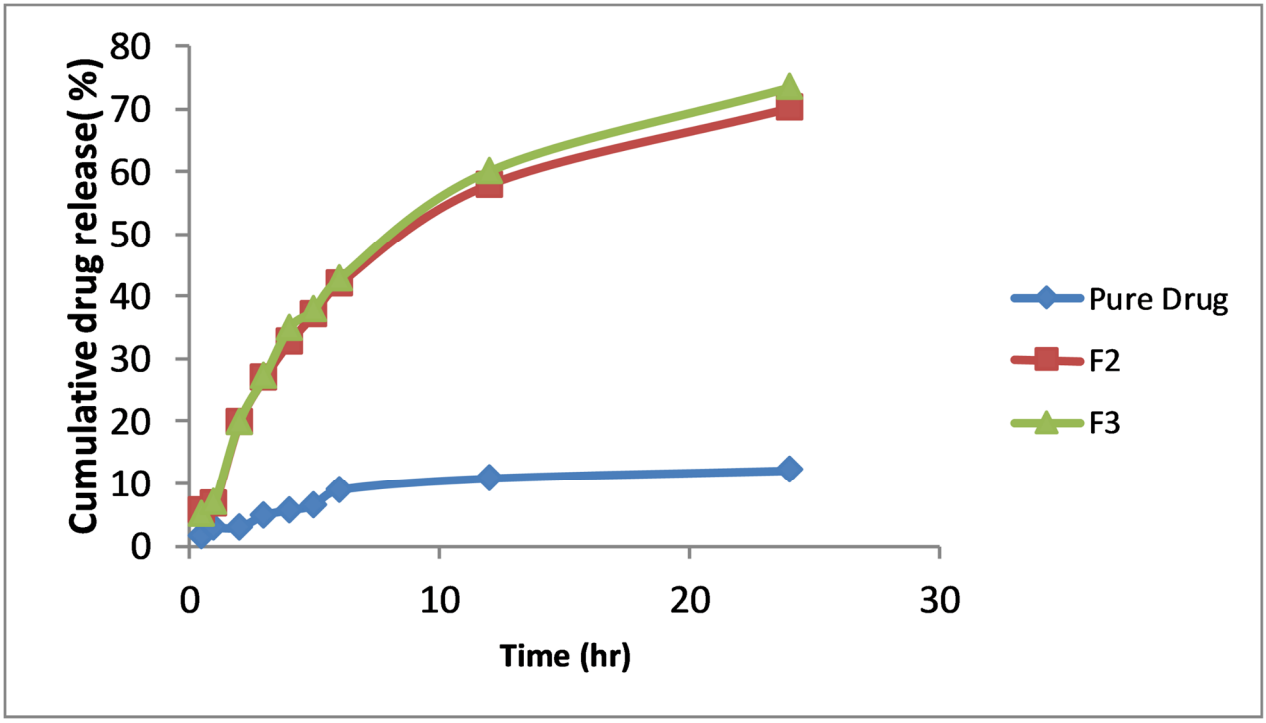

Fig. 5: *In vitro* dissolution of Celecoxib Pure Drug, F1 (f2) F (f3).

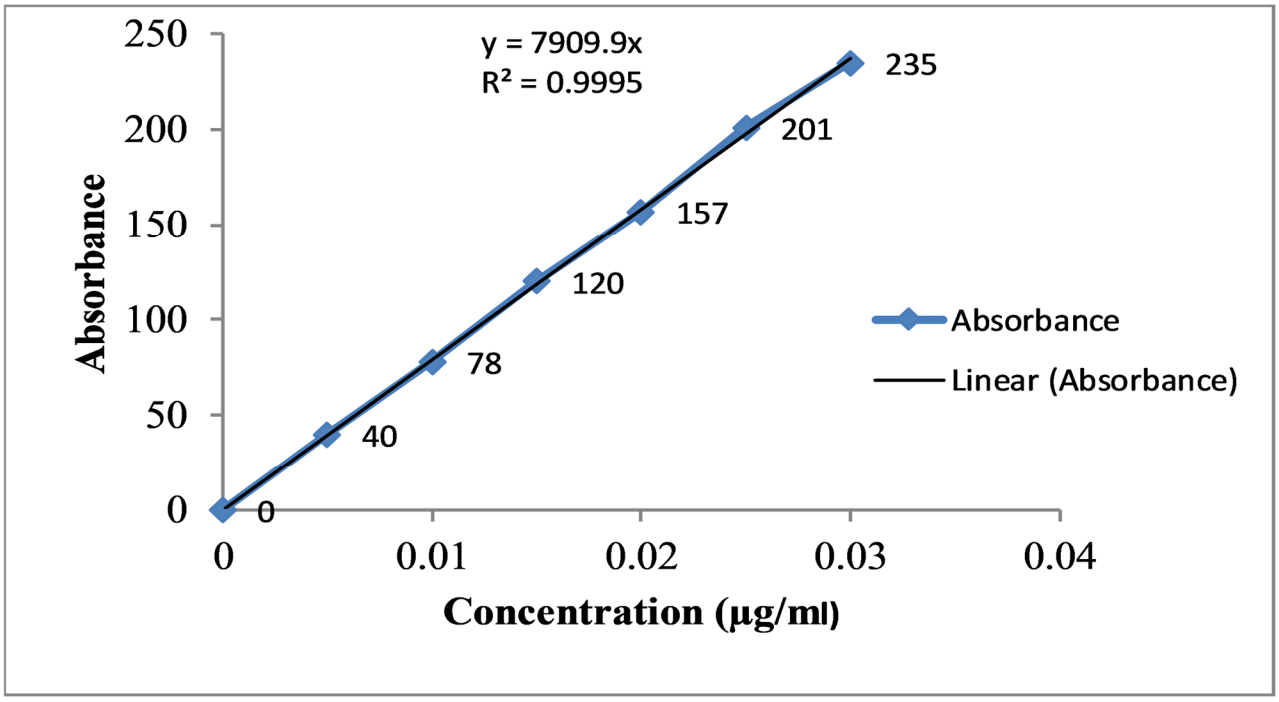

Fig. 6: Celecoxib standard curve varying concentrations

Table 1: Micro particle formulation

Formulation code	Celecoxib	HPMC	Droplet stabilizer	Surfactant	Swirling speed
F1	200(mg)	200 (mg)	100(mg)	1(%)	1000(rpm)
F2	200(mg)	500(mg)	100(mg)	1(%)	1000(rpm)

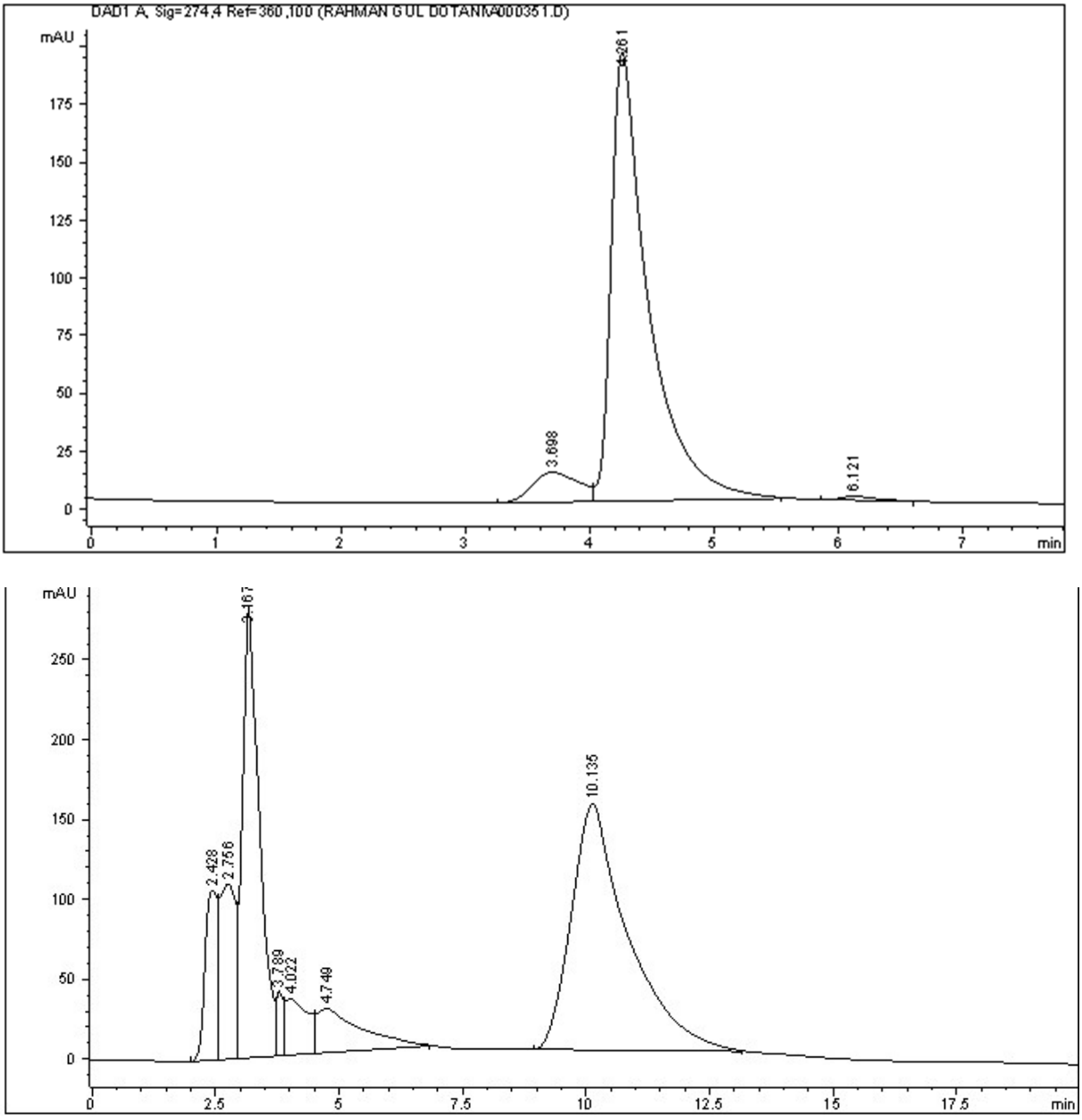

Fig. 7: Celecoxib chromatogram: (A) without and (B) with rabbit plasma.

Table 2: Accuracy and Intra-day and inter-day precision of the HPLC technique

Intra-day measured concentration (n = 5)					
S. No	Parameters	Nominal concentration (μg ml^{-1})	Mean concentration (μg ml^{-1}) ± SD	Precision (RSD %)	Accuracy (RSE %)
1	LQC	0.22	0.250+0.012	5.10	99.21
2	MQC	12.10	12.21+0.213	3.24	98.23
3	HQC	19.10	20.10+0.342	3.45	97.22
Inter-day measured concentration (n = 5)					
1	LQC	0.22	0.248+0.022	6.10	98.21
2	MQC	12.10	12.11+0.113	3.22	99.03
3	HQC	19.10	20.12+0.242	3.15	98.12

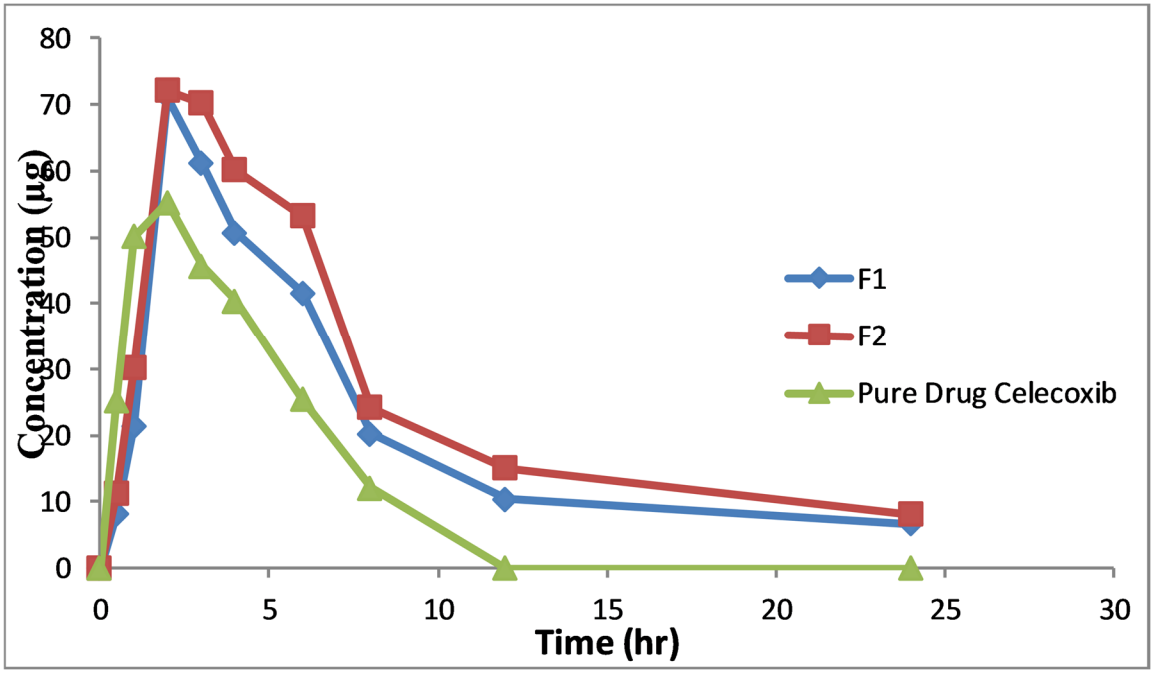

Fig. 8: A comparison of pure Celecoxib, F1 and F2" after oral administration in plasma concentration versus time profile.

Table 3: Celecoxib, F1 and F2, pharmacokinetic parameters mean values

S. No	Pharmacokinetic parameters	Units	Dose (2mg/Kg body weight)		
			Group-I Pure Celecoxib (Control)	Group-II Formulation (F1)	Group-III Formulation (F2)
1	C_{max}	µg/ml	35.337	42.135	45.236
2	T_{max}	H	3	3	2
3	$AUC_{0-\alpha}$	µg/ml*h	396.724	498.44	499.96
4	MRT	H	12.15	11.36	9.41
5	$t_{1/2}$	H	8.219	7.42	9.53

Celecoxib pharmacokinetic profile in rabbit plasma

To evaluate the isolation process, parameters such as selectivity, precision, accuracy, robustness and working range were examined using a validated HPLC method. For conducting the pharmacokinetic analysis, limits of detection (LOD) and quantification (LOQ) were also determined (Gul *et al*., 2019).

The pharmacokinetics of celecoxib were assessed in three independent groups of rabbits (n = 3 each) as also reported this method by (Nasar *et al*., 2020), following oral administration of either the pure drug or celecoxib-loaded HPMC microparticles (formulations F1 and F2) (Gul *et al*., 2019). Average plasma concentrations of celecoxib are shown in fig. 8. Statistical analysis using SPSS revealed that both formulations (F1 and F2) resulted in significantly higher plasma drug levels compared to the control group ($p < 0.05$). table 3 outlines the pharmacokinetic profiles and overall performance of the pure drug. The maximum plasma concentration (Cmax), representing the peak level of drug in systemic circulation post-oral administration, was found to be 35.337±0.17 µg/mL for the pure drug (Celecoxib), 42.135±1.12 µg/mL for F1 and 95.236±0.29 µg/mL for F2. These elevated Cmax values for the formulations indicate an enhancement in drug bioavailability.

Tmax, the time taken to reach Cmax, was recorded as 3±0.81 hours for pure celecoxib, 2±0.66 hours for F1 and 2±0.44 hours for F2, showing a quicker absorption rate for the formulations. The area under the plasma concentration-time curve (AUC), calculated using the trapezoidal rule based on statistical moment theory, reflects the extent of drug absorption. Mean $AUC_{0-\infty}$ values (±SD) were 396.724±10.34 µg/mL·h for celecoxib, 498.44±8.49 µg/mL·h for F1 and 499.96±6.15 µg/mL·h for F2. The ranking order of $AUC_{0-\infty}$ was F2 > F1 > pure celecoxib, confirming greater systemic exposure and bioavailability for the test formulations. The mean residence time (MRT), indicating the average time the drug remains in systemic circulation, was 12.15±3.72 h for the pure drug, 11.36±1.12 h for F1 and 9.41±4.19 h for F2. Finally, the elimination half-life (t½) was 8.219±0.77 h for pure celecoxib, 7.42±1.50 h for F1 and 0.63±1.55 h for F2. The

reduced half-life of F2, in particular, suggests a different drug release and clearance profile, possibly indicating improved control and efficiency of the drug delivery system *in vivo*. All the results were significant ($P<0.05$).

CONCLUSION

In this study, we report the effective development of HPMC-based microparticles containing celecoxib (formulations F1 and F2), produced through a solvent evaporation (ESE) oil-in-oil emulsion method using a hydrophilic polymer as the matrix in varying proportions. These microparticles were thoroughly analyzed for their physicochemical properties and their drug release behavior in vitro was examined. During the formulation process, celecoxib underwent a transition from its crystalline form to an amorphous state, as confirmed by differential scanning calorimetry (DSC) and X-ray diffraction (XRD) analyses. Altering the polymer concentration significantly influenced both the particle size and the overall drug release profile. Specifically, drug release was slower in acidic environments and more controlled in basic media when compared to the release from unformulated celecoxib. Moreover, in vivo studies conducted on healthy rabbits using the raw drug and both formulations demonstrated favorable pharmacokinetic profiles. These findings suggest that the designed microparticles have strong potential for enhancing the solubility and controlled delivery of poorly water-soluble drugs, particularly targeting release in the colon.

Conflict of interest

The authors declare no conflict of interest for this research work.

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