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# METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF DICLOFENAC SODIUM AND BENZYL ALCOHOL IN GEL FORMULATION

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**Abstract:**

*A precise, accurate, and stability-indicating reversed phase high performance liquid chromatography (RP-HPLC) method was created and approved for the simultaneous measurement of benzyl alcohol and diclofenac sodium in gel formulation. Potassium dihydrogen phosphate buffer and acetonitrile (60:40 v/v) were used as the mobile phase in a C18 column to accomplish chromatographic separation at a flow rate of 1.0 mL/min. The wavelength used for detection was 240 nm. For Diclofenac Sodium and Benzyl Alcohol, the developed method yielded symmetrical, well-resolved peaks with retention durations of 3.146 and 6.142 minutes. For linearity, accuracy, precision, specificity, robustness, ruggedness, LOD, and LOQ, the method was validated in accordance with ICH requirements. For both analytes, correlation coefficient values were more than 0.998. Recovery studies showed adequate percentage recovery values and great precision. The technique was effectively used to analyze commercial gel compositions for routine quality control.*

***Keywords:*** *gel formulation, simultaneous estimation, pharmaceutical analysis, Diclofenac sodium, benzyl alcohol, RP-HPLC, stability-indicating method, and method validation*

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## INTRODUCTION:

### Pharmaceutical Analytical Chemistry

The identification, characterisation, quantification, and purity assessment of pharmaceutical compounds and dosage forms are the focus of the specialist field of pharmaceutical analytical chemistry. From drug discovery and formulation development to manufacturing, quality control, stability testing, and regulatory approval, it is essential to a pharmaceutical product's lifespan. The accuracy and precision of the analytical techniques used for their production are crucial to the dependability and safety of medications.[1]
assessment.[2]

The rapid growth of pharmaceutical sciences has resulted in the development of complex drug molecules and sophisticated dosage forms. Consequently, conventional analytical methods are no longer sufficient to meet modern analytical requirements. Advanced analytical techniques provide improved sensitivity, selectivity, reproducibility, and automation, making them suitable for compliance with regulatory standards established by ICH, USP, BP, and IP.[3]

### Importance of Analytical Methods in Pharmaceutical Quality Assurance

Analytical methods play an important role in pharmaceutical quality assurance by ensuring that medicinal products consistently meet safety and efficacy standards. Reliable analytical procedures provide scientific evidence regarding the quality, purity, and stability of pharmaceutical products.[4]
Analytical techniques are extensively used for identification testing, assay determination, impurity profiling, dissolution studies, content uniformity testing, and stability evaluation of pharmaceutical dosage forms.[5] Improper or poorly validated analytical procedures may produce inaccurate results, which can compromise patient safety and regulatory compliance.[6] Therefore, analytical methods must be scientifically sound, precise, accurate, robust, and validated according to regulatory guidelines.[7]

### Classification of Analytical Methods

Analytical methods used in pharmaceutical sciences are broadly classified into classical methods and instrumental methods. Classical analytical methods include titrimetric, gravimetric, and colorimetric techniques. These methods are simple and economical but possess limitations such as low sensitivity and greater chances of human error.[8]
Instrumental analytical methods involve the use of sophisticated instruments to measure physical or chemical properties of analytes. These methods provide improved accuracy, precision, sensitivity, and reproducibility compared to conventional analytical approaches.[9] Common instrumental techniques include UV–Visible spectroscopy, infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectrometry, and chromatographic techniques.[10]

### Chromatography

Chromatography is one of the most important analytical techniques employed in pharmaceutical analysis for the separation, identification, and quantification of components present in a mixture. The principle of chromatography is based on the differential distribution of analytes between a stationary phase and a mobile phase.[11]

Different components of a mixture interact differently with these phases and migrate at different rates, resulting in separation. Chromatographic techniques are highly useful in pharmaceutical analysis because formulations often contain multiple components such as active ingredients, excipients, preservatives, impurities, and degradation products.[12]

Chromatography is widely used in pharmaceutical quality control laboratories for assay determination, impurity profiling, dissolution studies, stability testing, and preservative analysis.[13] Advancements in chromatographic techniques have resulted in improved resolution, faster analysis, and enhanced sensitivity.[14]

### Classification of Chromatography

Chromatographic techniques may be classified based on the physical state of the mobile phase, separation mechanism, or polarity of phases. Based on the physical state of the mobile phase, chromatography is classified into gas chromatography, liquid chromatography, and supercritical fluid chromatography.[15]

Gas chromatography uses a gaseous mobile phase and is suitable for volatile and thermally stable compounds.[16] Liquid chromatography employs a liquid mobile phase and is widely used for non-volatile and thermally unstable compounds.[17] Supercritical fluid chromatography combines the advantages of gas and liquid chromatography and utilizes supercritical fluids such as carbon dioxide as the mobile phase.[18]

Based on the separation mechanism, chromatography is classified into adsorption chromatography, partition chromatography, ion exchange chromatography, size exclusion chromatography, and affinity chromatography.[19] Adsorption chromatography involves separation due to adsorption of analytes on a solid stationary phase.[20] Partition chromatography is based on

partitioning of analytes between two immiscible liquid phases.[21] Ion exchange chromatography involves electrostatic interaction between charged analytes and ion exchange resins.[22] Size exclusion chromatography separates compounds based on molecular size differences.[23] Affinity chromatography depends upon specific biological interactions between analytes and the stationary phase.[24]

According to the polarity of phases, chromatography is classified into normal phase chromatography and reversed phase chromatography.[25] In normal phase chromatography, the stationary phase is polar while the mobile phase is non-polar.[26] In reversed phase chromatography, the stationary phase is non-polar whereas the mobile phase is polar.[27] Reversed phase chromatography is the most commonly used mode in pharmaceutical analysis due to its broad applicability and compatibility with aqueous systems.[28]

**High Performance Liquid Chromatography**

High Performance Liquid Chromatography (HPLC) is an advanced form of liquid chromatography that employs high pressure to pass the mobile phase through a column packed with finely divided stationary phase particles.[29] HPLC is widely used in pharmaceutical industries because of its high sensitivity, precision, reproducibility, and rapid analytical capability.[30]

The principle of HPLC is based on the differential interaction of analytes between the stationary phase and mobile phase. Components with weaker interaction elute faster, whereas those with stronger interaction are retained longer, resulting in separation.[31] Factors such as mobile phase composition, flow rate, stationary phase characteristics, and column temperature significantly influence chromatographic separation.[32]

**Reversed Phase High Performance Liquid Chromatography**

Reversed Phase High Performance Liquid Chromatography (RP-HPLC) is the most widely employed mode of HPLC in pharmaceutical analysis. In RP-HPLC, the stationary phase is non-polar, generally consisting of C18 or C8 bonded silica, while the mobile phase is relatively polar and consists of water mixed with organic solvents such as methanol or acetonitrile.[33]

RP-HPLC offers several advantages including excellent reproducibility, compatibility with aqueous samples, high resolution, and ease of method development.[34] It is extensively used for assay determination, impurity profiling, dissolution studies, and stability analysis of pharmaceutical formulations.[35]

Diclofenac Sodium is a widely used non-steroidal anti-inflammatory drug possessing potent analgesic and anti-inflammatory properties. Benzyl Alcohol is commonly used as a preservative in pharmaceutical gel formulations to prevent microbial contamination and improve product stability.[36] Accurate and reliable simultaneous estimation of Diclofenac Sodium and Benzyl Alcohol is therefore essential to ensure product quality, safety, efficacy, and regulatory compliance.[37]

**Chromatographic Parameters**

Chromatographic parameters are quantitative measures used to evaluate the efficiency, performance, and reliability of a chromatographic system. These parameters are essential during method development, optimization, and validation because they directly influence the accuracy, precision, and reproducibility of analytical results.[38]

**Retention Time (Rt)**

Retention time is the time required for an analyte to travel through the chromatographic column and reach the detector after sample injection. It is an important parameter used for qualitative identification of compounds. Retention time is influenced by factors such as mobile phase composition, flow rate, stationary phase characteristics, and column temperature.[39] Consistent retention time indicates stable chromatographic conditions, whereas significant variation may suggest system instability or column deterioration.[40]

**Capacity Factor (k′)**

The capacity factor, also known as retention factor, indicates the extent of retention of an analyte on the chromatographic column relative to the mobile phase.[41] It provides information regarding the interaction between the analyte and stationary phase. An ideal capacity factor generally ranges between 1 and 10 for efficient chromatographic separation.[42]

**Selectivity (α)**

Selectivity represents the ability of the chromatographic system to separate two closely eluting analytes. It is expressed as the ratio of capacity factors of two compounds.[43] Higher selectivity values indicate better separation efficiency between adjacent peaks.[44] Mobile phase composition, pH, and stationary phase properties significantly affect selectivity.[45]

**Resolution (Rs)**

Resolution is an important chromatographic parameter that describes the degree of separation

between two adjacent peaks. Adequate resolution is necessary for accurate identification and quantification of analytes in pharmaceutical formulations.[46] A resolution value greater than 2 is generally considered acceptable for complete peak separation.[47]

**Column Efficiency**

Column efficiency is expressed in terms of the number of theoretical plates (N) and indicates the separating capability of the chromatographic column.[48] Higher theoretical plate values indicate better column performance and sharper chromatographic peaks.[49] Efficient columns improve sensitivity, reproducibility, and peak resolution.[50]

**Peak Asymmetry and Tailing Factor**

Peak asymmetry or tailing factor describes the symmetry of chromatographic peaks. Ideally, chromatographic peaks should be symmetrical; however, peak tailing or fronting may occur due to secondary interactions, inappropriate mobile phase composition, or column overloading.[51] For acceptable chromatographic performance, the tailing factor should generally be less than 2.[52]

**Factors Affecting Chromatographic Parameters**

Several experimental variables influence chromatographic parameters and overall system performance. These factors include mobile phase composition, pH, flow rate, column dimensions, particle size, temperature, and sample concentration.[53] Proper optimization of these variables is essential to achieve reliable and reproducible chromatographic results.[54]

**Importance in Method Development**

Chromatographic parameters play a vital role during method development and validation. They help in assessing system suitability, improving resolution, ensuring reproducibility, and establishing robustness of the analytical method.[55] Regulatory guidelines recommend routine monitoring of chromatographic parameters to ensure consistent analytical performance and reliable pharmaceutical analysis.[56]

**DRUG PROFILE**

**Diclofenac Sodium**

Diclofenac Sodium is a non-steroidal anti-inflammatory drug (NSAID) widely used for its analgesic, anti-inflammatory, and antipyretic properties. It is commonly prescribed for the treatment of pain, inflammation, arthritis, and musculoskeletal disorders. Diclofenac Sodium is frequently formulated as topical gels to provide localized therapeutic action with reduced systemic side effects.[57]

The drug possesses aromatic rings and functional groups that exhibit significant UV absorption, making it suitable for analysis by RP-HPLC with UV detection. Diclofenac Sodium is slightly soluble in water and freely soluble in methanol, which facilitates its extraction and chromatographic analysis from gel formulations.[58]

**Benzyl Alcohol**

Benzyl Alcohol is a pharmaceutical excipient commonly used as a preservative in topical and injectable formulations. It possesses antimicrobial properties that help prevent microbial contamination and improve formulation stability. In pharmaceutical gels, Benzyl Alcohol plays an important role in maintaining product safety and shelf life.

Benzyl Alcohol also shows UV absorbance and can be simultaneously estimated along with active pharmaceutical ingredients using RP-HPLC methods. Accurate estimation of Benzyl Alcohol is necessary to ensure preservative levels remain within acceptable regulatory limits.[59]

Simultaneous estimation of Diclofenac Sodium and Benzyl Alcohol is important for ensuring therapeutic efficacy, product quality, and microbial safety of gel formulations. RP-HPLC provides adequate selectivity, sensitivity, and reproducibility for simultaneous analysis of both components without interference from excipients.[60]

**Table 1: Physicochemical Properties of Diclofenac Sodium and Benzyl Alcohol**

| Parameter | Diclofenac Sodium | Benzyl Alcohol |
|---|---|---|
| Chemical category | NSAID | Preservative |
| Molecular formula | $C_{14}H_{10}Cl_2NNaO_2$ | $C_7H_8O$ |
| Molecular weight | 318.13 g/mol | 108.14 g/mol |
| Solubility | Slightly soluble in water, soluble in methanol | Soluble in water and alcohol |
| UV absorption | Shows UV absorbance | Shows UV absorbance |
| Analytical relevance | Active drug estimation | Preservative estimation |

**Analytical Relevance of Selected Drugs**

Diclofenac Sodium and Benzyl Alcohol were selected for the present study because of their widespread use in topical gel formulations and the need for reliable analytical methods for quality control and regulatory compliance. Their physicochemical and chromatographic properties make them suitable candidates for simultaneous estimation using RP-HPLC.[61]

## MATERIALS AND METHODS:

### Selection of Drug

Diclofenac Sodium was selected as the active pharmaceutical ingredient because of its extensive use as a non-steroidal anti-inflammatory drug in topical gel formulations. Benzyl Alcohol was selected as the preservative due to its antimicrobial properties and common use in pharmaceutical gels. Simultaneous estimation of both components is essential for ensuring therapeutic efficacy and formulation safety.[62]

### Procurement of Drug and Chemicals

All drugs, chemicals, and reagents used in the study were procured from approved sources. Analytical reagent grade chemicals and HPLC grade solvents were used throughout the experimental work.

**Table 2: List of Drugs Used**

| Sr. No. | Name | Category | Source |
|---|---|---|---|
| 1 | Diclofenac Sodium | Active Pharmaceutical Ingredient | Indo Pharma, Mumbai |
| 2 | Benzyl Alcohol | Preservative | Indo Pharma, Mumbai |
| 3 | Pain Win Gel | Marketed Formulation | Super Formulation Pvt. Ltd. |

**Table 3: Chemicals and Reagents Used**

| Sr. No. | Chemical/Reagent | Grade | Role |
|---|---|---|---|
| 1 | Methanol | HPLC Grade | Mobile phase |
| 2 | Acetonitrile | HPLC Grade | Mobile phase |
| 3 | Potassium Dihydrogen Phosphate | AR Grade | Buffer |
| 4 | Orthophosphoric Acid | AR Grade | pH adjustment |

### Instruments and Apparatus

All instruments used during the study were calibrated and maintained under standard laboratory conditions to ensure reliability and reproducibility of analytical results.

**Table 4: List of Instruments Used**

| Sr. No. | Instrument |
|---|---|
| 1 | FTIR Spectrophotometer |
| 2 | UV–Visible Spectrophotometer |
| 3 | HPLC System with UV Detector |
| 4 | Analytical Balance |
| 5 | Ultrasonic Bath |

### Physicochemical Characterization

Diclofenac Sodium was evaluated for physical appearance and solubility behavior to support analytical method development. The drug appeared as a white crystalline powder and showed good solubility in methanol, indicating its suitability for RP-HPLC analysis.[63–64]

**Table 5: Physicochemical Properties of Diclofenac Sodium**

| Parameter | Observation |
|---|---|
| Appearance | White crystalline powder |
| Solubility | Soluble in methanol |
| Category | NSAID |
| Analytical suitability | Suitable for RP-HPLC |

### Preparation of Buffer Solution

Potassium dihydrogen phosphate buffer was prepared in distilled water, and pH was adjusted using orthophosphoric acid. The solution was filtered and degassed before use in chromatographic analysis.[65]

### Drug–Excipient Compatibility Study

FTIR spectroscopy was used to study compatibility between Diclofenac Sodium and Benzyl Alcohol. The absence of major changes in characteristic peaks indicated compatibility between drug and preservative.[66]

### Development of RP-HPLC Method

The RP-HPLC method was developed to achieve effective separation of Diclofenac Sodium and Benzyl Alcohol with good resolution and peak symmetry. A C18 column (250 mm × 4.6 mm, 5 µm) was selected for analysis.[67]

Different mobile phase combinations were evaluated, and potassium dihydrogen phosphate buffer with acetonitrile (60:40 v/v) was found suitable for optimum separation. A flow rate of 1.0 mL/min and detection wavelength of 240 nm were selected for analysis.[68]

**Table 6: Optimized Chromatographic Conditions**

| Parameter | Condition |
|---|---|
| Column | C18 RP Column |
| Mobile phase | Buffer : Acetonitrile (60:40) |
| Flow rate | 1.0 mL/min |
| Detection wavelength | 240 nm |
| Injection volume | 20 µL |
| Elution mode | Isocratic |

**Preparation of Standard Solutions**

Standard stock solutions of Diclofenac Sodium and Benzyl Alcohol were prepared separately in methanol to obtain concentrations of 1000 µg/mL. Working standard solutions were prepared by suitable dilution with mobile phase.[69–70]

**Calibration Curve Preparation**

Calibration curves were prepared by injecting working standard solutions of different concentrations into the HPLC system under optimized chromatographic conditions. Peak area versus concentration plots were constructed for both analytes.[71]

**System Suitability Test**

System suitability testing was performed before analysis to ensure adequate chromatographic performance. Parameters such as retention time, theoretical plates, peak area, and tailing factor were evaluated according to ICH guidelines.[72]

**Table 7: System Suitability Parameters**

| Parameter | Acceptance Criteria |
|---|---|
| %RSD of peak area | ≤ 2.0 |
| Tailing factor | ≤ 2.0 |
| Theoretical plates | ≥ 2000 |

**Method Validation**

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines for parameters including linearity, accuracy, precision, LOD, LOQ, robustness, and ruggedness.[73]

Linearity was evaluated by regression analysis of calibration curves, and good correlation coefficients confirmed linear response.[74] Accuracy was determined by recovery studies using the standard addition method.[75] Precision studies showed low %RSD values, indicating good reproducibility of the method.[76]

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated using standard deviation and slope of the calibration curve, indicating the sensitivity of the method.[77] Robustness and ruggedness studies demonstrated reliability of the developed RP-HPLC method under varied analytical conditions.[78]

## RESULTS AND DISCUSSION:

**FTIR Analysis Study**

Fourier Transform Infrared (FTIR) spectroscopy was performed to evaluate the compatibility between Diclofenac Sodium and Benzyl Alcohol. The FTIR spectrum of Diclofenac Sodium showed characteristic peaks corresponding to aromatic C–H stretching, carboxylate group vibrations, and secondary amine groups, confirming its chemical identity and purity.

Benzyl Alcohol exhibited characteristic absorption bands corresponding to hydroxyl (O–H) stretching and aromatic C–H vibrations, confirming its identity.

The FTIR spectrum of the physical mixture showed all major characteristic peaks of both Diclofenac Sodium and Benzyl Alcohol without significant shifting or disappearance of peaks. This indicates the absence of chemical interaction between the drug and preservative, confirming their compatibility for formulation and RP-HPLC analysis.

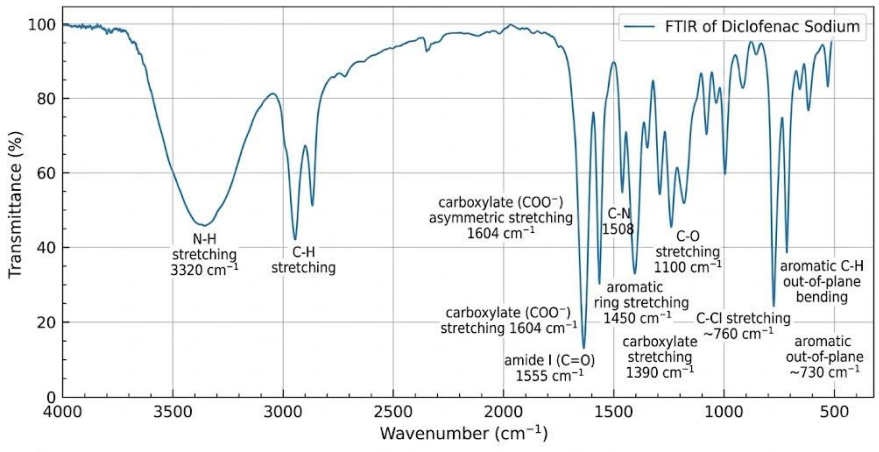


**Figure 1:** FTIR spectrum of Diclofenac Sodium

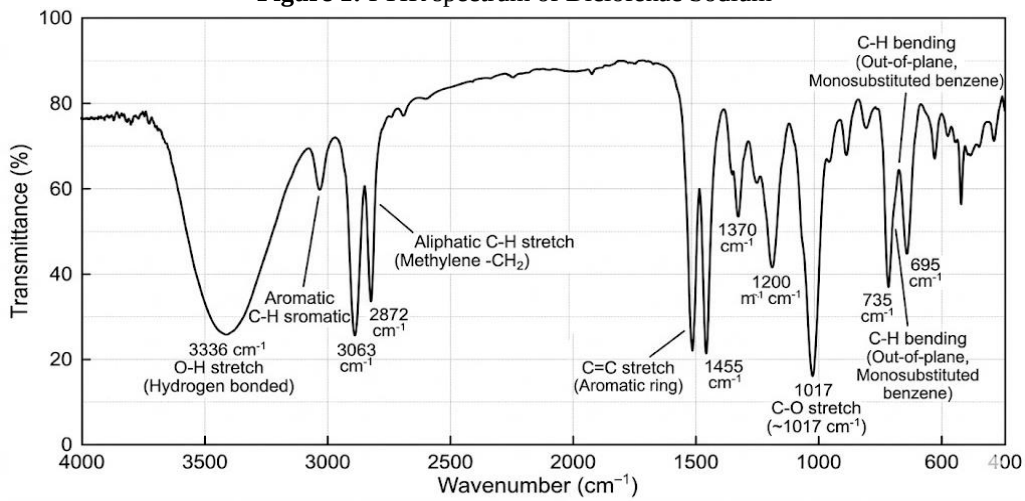


**Figure 2:** FTIR spectrum of Benzyl Alcohol

Figure X: FTIR spectrum of physical mixture of Diclofenac Sodium and Benzyl Alcohol.

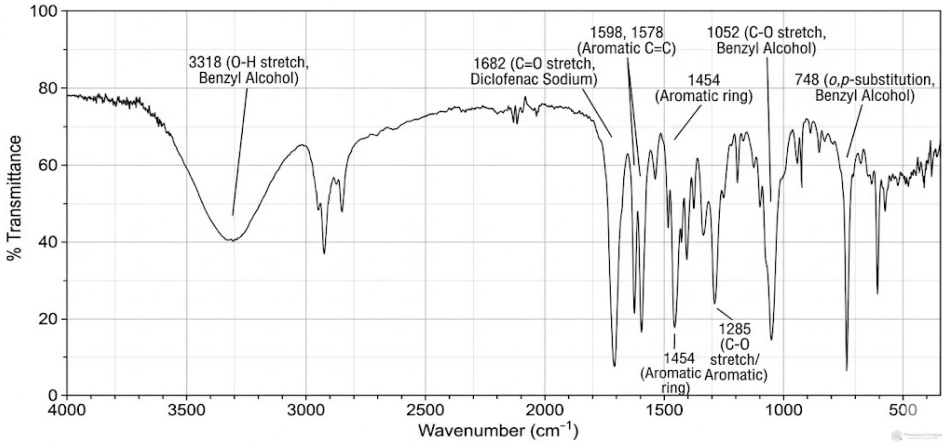


**Figure 3:** FTIR spectrum of physical mixture of Diclofenac Sodium and Benzyl Alcohol

**UV–Visible Spectrophotometric Analysis**

UV–Visible spectrophotometric analysis was performed for selection of detection wavelength. Standard solutions of Diclofenac Sodium and Benzyl Alcohol were scanned in the range of 200–400 nm.

Diclofenac Sodium showed maximum absorbance at 240 nm, while Benzyl Alcohol also exhibited adequate absorbance at the same wavelength. Therefore, 240 nm was selected for simultaneous RP-HPLC analysis of both analytes without spectral interference.

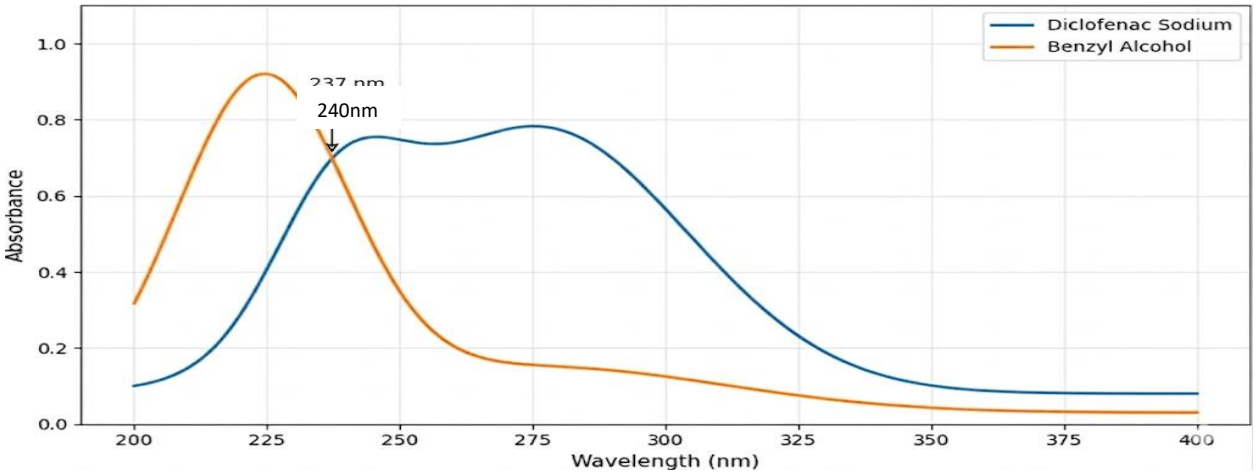


**Figure 4:** Overlay UV spectrum of Diclofenac Sodium and Benzyl Alcohol

**RP-HPLC Method Development Study**

Chromatographic separation of Diclofenac Sodium and Benzyl Alcohol was successfully achieved using a C18 RP-HPLC column with potassium dihydrogen phosphate buffer and acetonitrile (60:40 v/v, pH 3.0).

Well-resolved and symmetrical peaks were obtained for both analytes without interference from excipients, indicating good selectivity of the method. A flow rate of 1.0 mL/min and detection wavelength of 240 nm provided satisfactory resolution and reproducible chromatographic performance.

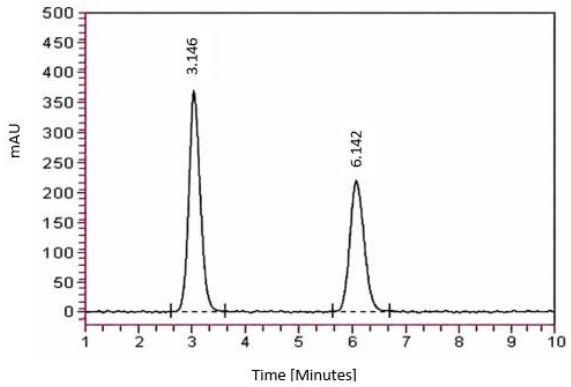


**Figure 5:** Optimized RP-HPLC chromatogram of Diclofenac Sodium and Benzyl Alcohol

**System Suitability Study**

System suitability testing was performed by injecting a mixed standard solution of Diclofenac Sodium and Benzyl Alcohol six times under optimized chromatographic conditions.

Parameters such as retention time, peak area, theoretical plates, and tailing factor were evaluated. The %RSD values were within acceptable limits, indicating good repeatability. Theoretical plate count and tailing factor values confirmed adequate column efficiency and symmetrical peak shape, demonstrating suitability of the RP-HPLC system for analysis.

**Table 8: System Suitability Parameters**

| Parameter | Diclofenac Sodium | Benzyl Alcohol | Acceptance Criteria |
|---|---|---|---|
| Retention time (min) | 3.146 | 6.142 | Consistent |
| %RSD of peak area | 0.84 | 0.92 | ≤ 2.0 |
| Tailing factor | 1.21 | 1.18 | ≤ 2.0 |
| Theoretical plates | 6540 | 4875 | ≥ 2000 |

**7.5 Linearity Study**

Linearity was evaluated to establish the proportional relationship between analyte concentration and detector response. Diclofenac Sodium and Benzyl Alcohol standard solutions were prepared at six concentration levels within the analytical range and injected in triplicate.

Calibration curves were constructed by plotting mean peak area versus corresponding concentration. Linear regression analysis demonstrated a linear response over the selected concentration range for both analytes. The high correlation coefficient values indicate good linearity and suitability of the method for quantitative estimation.

**Table 9: Linearity Data for Diclofenac Sodium**

| Concentration (µg/mL) | Mean Peak Area |
|---|---|
| 10 | 152346 |
| 20 | 301824 |
| 30 | 456210 |
| 40 | 608742 |
| 50 | 761435 |
| 60 | 914862 |

Correlation coefficient ($r^2$) = **0.9992**

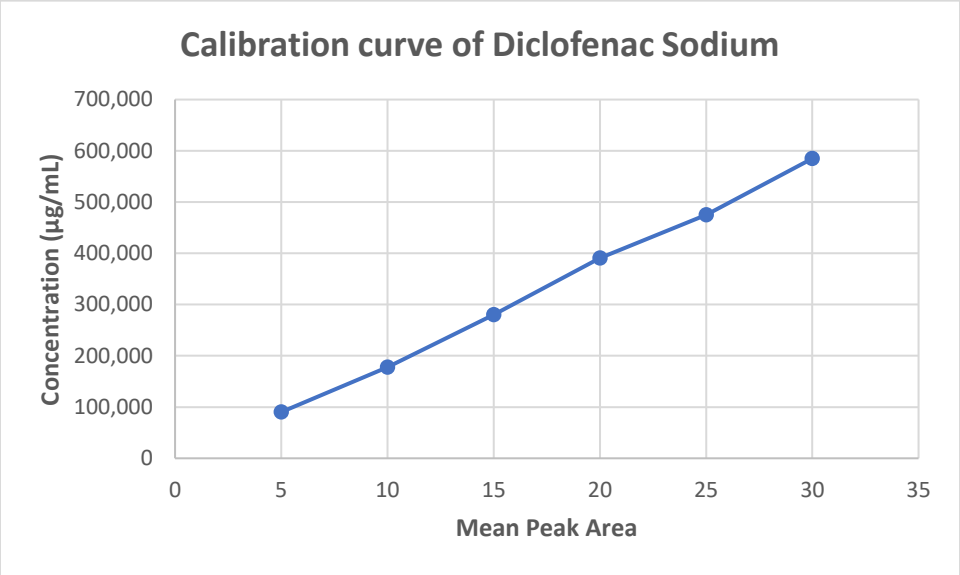


**Figure 6: Calibration curve of Diclofenac Sodium**

**Table 10: Linearity Data for Benzyl Alcohol**

| Concentration (µg/mL) | Mean Peak Area |
|---|---|
| 5 | 98214 |
| 10 | 197540 |
| 15 | 296183 |
| 20 | 395764 |
| 25 | 495182 |
| 30 | 594936 |

Correlation coefficient ($r^2$) = **0.9989**

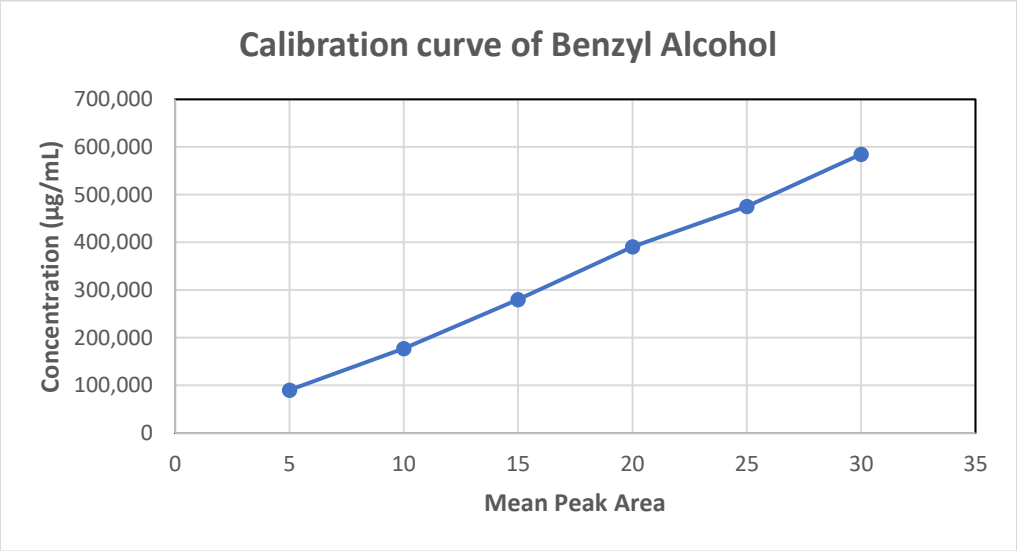


**Figure 7: Calibration curve of Benzyl Alcohol**

**7.6 Accuracy Study**

Accuracy of the method was evaluated by recovery studies using the standard addition technique at three concentration levels: 80%, 100%, and 120%. Known quantities of Diclofenac Sodium and Benzyl Alcohol were added to the pre-analyzed sample and analyzed under optimized chromatographic conditions.

The percentage recovery values obtained were within acceptable limits, confirming the accuracy of the method and absence of interference from formulation excipients.

**Table 11: Accuracy Study for Diclofenac Sodium**

| Level (%) | Amount Added (µg/mL) | Amount Recovered (µg/mL) | % Recovery |
|---|---|---|---|
| 80 | 24 | 23.86 | 99.42 |
| 100 | 30 | 29.91 | 99.70 |
| 120 | 36 | 36.18 | 100.50 |

**Table 12: Accuracy Study for Benzyl Alcohol**

| Level (%) | Amount Added (µg/mL) | Amount Recovered (µg/mL) | % Recovery |
|---|---|---|---|
| 80 | 12 | 11.91 | 99.25 |
| 100 | 15 | 14.87 | 99.13 |
| 120 | 18 | 18.09 | 100.50 |

**7.7 Precision Study**

Precision of the analytical method was evaluated in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). Multiple injections of the same concentration were analyzed on the same day and on different days.

Precision was expressed as %RSD of peak area values. Low %RSD values indicate good precision and reproducibility of the method.

**Table 13: Intra-day Precision Study**

| Injection No. | Diclofenac Sodium Peak Area | Benzyl Alcohol Peak Area |
|---|---|---|
| 1 | 456210 | 296183 |
| 2 | 458114 | 297024 |
| 3 | 455896 | 295742 |
| 4 | 457632 | 296510 |
| 5 | 456982 | 296891 |

%RSD

- Diclofenac Sodium = **0.41%**
- Benzyl Alcohol = **0.46%**

**Table 14: Inter-day Precision Study**

| Day | Diclofenac Sodium Peak Area | Benzyl Alcohol Peak Area |
|---|---|---|
| Day 1 | 456210 | 296183 |
| Day 2 | 458736 | 297412 |
| Day 3 | 455984 | 295906 |

%RSD

- Diclofenac Sodium = **0.53%**
- Benzyl Alcohol = **0.61%**

**Specificity Study**

Specificity of the developed RP-HPLC method was evaluated using blank, placebo, standard, and sample solutions under optimized chromatographic conditions.

No interfering peaks were observed in blank and placebo chromatograms at the retention times of Diclofenac Sodium and Benzyl Alcohol. Well-resolved peaks were obtained in standard and sample chromatograms, confirming the specificity of the method for simultaneous estimation of both analytes in gel formulation.

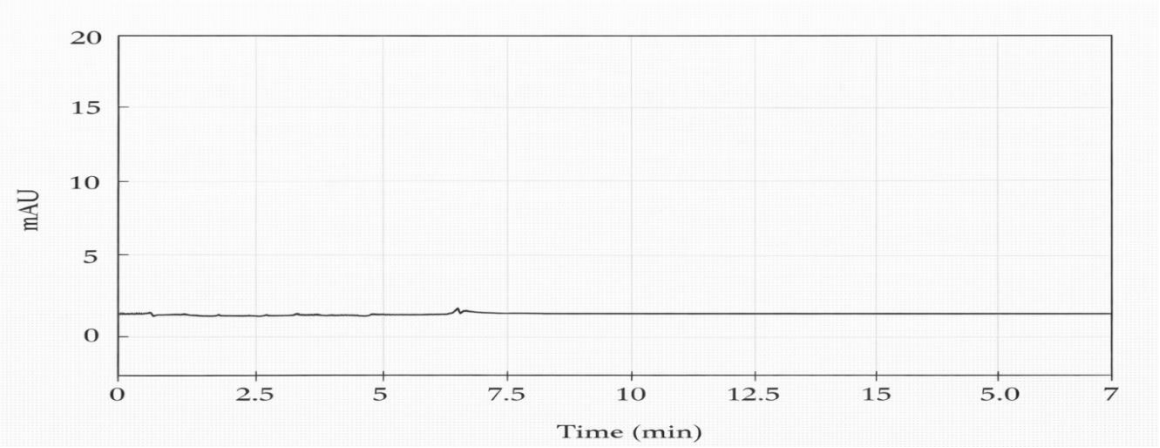


**Figure 8:** Chromatogram of blank solution

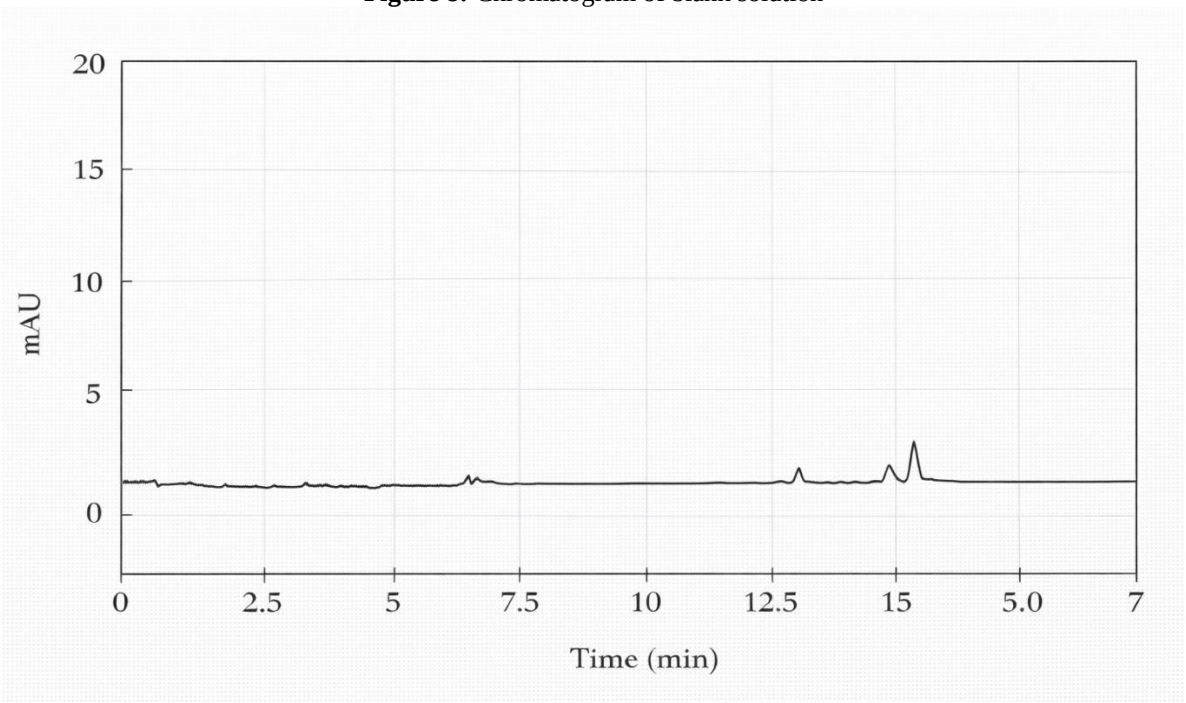


**Figure 7.9:** Chromatogram of placebo solution**.**

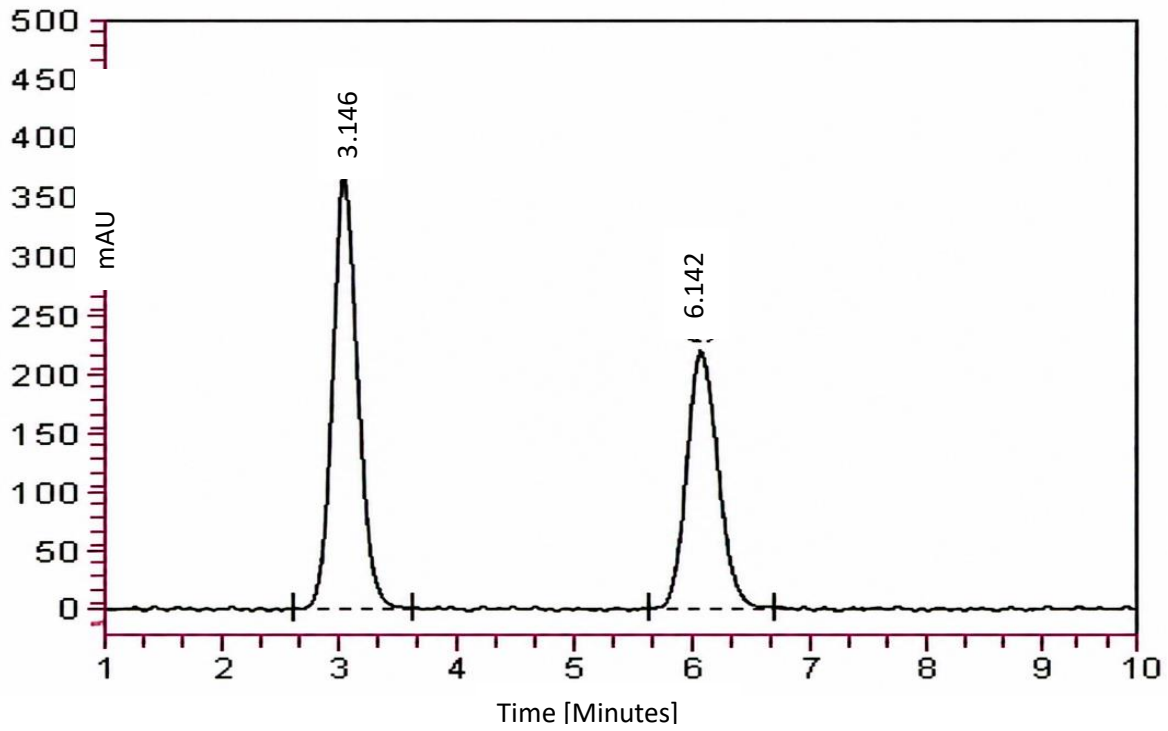


**Figure 10:** Chromatogram of sample solution

**7.9 Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

The sensitivity of the analytical method was evaluated by determining the limit of detection (LOD) and limit of quantitation (LOQ) based on the standard deviation of the response and the slope of the calibration curve, in accordance with ICH guidelines.

The calculated LOD values represent the lowest concentration of analyte that can be detected, while LOQ values indicate the lowest concentration that can be quantified with acceptable precision and accuracy. The obtained values demonstrate adequate sensitivity of the developed RP-HPLC method for routine quality control analysis.

**Table 15: LOD and LOQ Values**

| **Analyte** | **LOD (µg/mL)** | **LOQ (µg/mL)** |
|---|---|---|
| Diclofenac Sodium | 0.28 | 0.85 |
| Benzyl Alcohol | 0.19 | 0.58 |

**7.10 Robustness Study**

Robustness of the method was evaluated by introducing small deliberate variations in chromatographic conditions, including flow rate and mobile phase composition. The effect of these changes on retention time and peak area was studied.

Minor variations in flow rate (±0.1 mL/min) and mobile phase composition (±2%) did not produce significant changes in chromatographic behavior. The %RSD values remained within acceptable limits, indicating that the method is robust and reliable under normal laboratory variations.

**Table 16: Robustness Study**

| **Parameter Variation** | **%RSD (Diclofenac Sodium)** | **%RSD (Benzyl Alcohol)** |
|---|---|---|
| Flow rate (0.9 mL/min) | 0.78 | 0.82 |
| Flow rate (1.1 mL/min) | 0.85 | 0.89 |
| Mobile phase (58:42) | 0.92 | 0.96 |
| Mobile phase (62:38) | 0.88 | 0.91 |

**7.11 Ruggedness Study**

Ruggedness of the analytical method was assessed by performing the analysis under different conditions such as different analysts and different days. The consistency of chromatographic results under these variations was evaluated.

The %RSD values obtained for peak areas under different conditions were within acceptable limits, confirming the ruggedness and reproducibility of the developed method.

**Table 17: Ruggedness Study**

| Condition | %RSD (Diclofenac Sodium) | %RSD (Benzyl Alcohol) |
|---|---|---|
| Analyst 1 | 0.64 | 0.69 |
| Analyst 2 | 0.71 | 0.76 |
| Day 1 | 0.68 | 0.73 |
| Day 2 | 0.75 | 0.79 |

**7.12 Assay of Marketed Gel Formulation**

The validated RP-HPLC method was applied for the assay of Diclofenac Sodium and Benzyl Alcohol in marketed gel formulation. Sample solution prepared from the gel formulation was analyzed under optimized chromatographic conditions.

The assay values obtained were close to the labeled claim, indicating uniform drug content and confirming the applicability of the developed method for routine quality control analysis of gel formulations.

**Table 18: Assay of Marketed Gel Formulation**

| Component | Labeled Claim | Amount Found | % Assay |
|---|---|---|---|
| Diclofenac Sodium | 1.0% w/w | 0.99% w/w | 99.2 |
| Benzyl Alcohol | 1.0% w/w | 1.01% w/w | 101.0 |

**FUTURE PROSPECTS:**

The developed RP-HPLC method has significant potential for routine quality control analysis of Diclofenac Sodium and Benzyl Alcohol in pharmaceutical industries. Due to its simplicity, accuracy, precision, and reproducibility, the method can be effectively used for regular batch analysis of gel formulations to ensure product quality, safety, and consistency. The method may also be extended to other topical dosage forms such as creams, ointments, lotions, and transdermal preparations containing Diclofenac Sodium and Benzyl Alcohol. In future studies, the method can be applied for detailed stability testing and forced degradation studies under different environmental conditions such as temperature, humidity, and light exposure. This will help evaluate degradation behavior and confirm the stability-indicating nature of the analytical method. With advancements in analytical instrumentation, the method may further be adapted for automated and high-throughput analysis in industrial laboratories, reducing analysis time and increasing productivity.

The validated method can also support regulatory submissions and compliance with ICH guidelines. Additionally, it may serve as a reference analytical method for academic research and future studies involving similar drug–preservative combinations. Future research may also focus on developing greener analytical approaches by reducing solvent consumption and minimizing environmental impact while maintaining analytical efficiency and reproducibility.

## SUMMARY AND CONCLUSION:

The present study was carried out to develop and validate a simple, accurate, precise, and economical RP-HPLC method for simultaneous estimation of Diclofenac Sodium and Benzyl Alcohol in topical gel formulation. Diclofenac Sodium is widely used as a non-steroidal anti-inflammatory drug, while Benzyl Alcohol acts as a preservative in pharmaceutical preparations. Simultaneous estimation of both components is essential for ensuring quality, safety, and therapeutic effectiveness of the formulation.

FTIR compatibility studies confirmed the absence of interaction between Diclofenac Sodium and Benzyl Alcohol. UV spectrophotometric analysis identified 240 nm as the suitable detection wavelength for simultaneous analysis. The RP-HPLC method was successfully developed using a C18 column with potassium dihydrogen phosphate buffer and acetonitrile (60:40 v/v) as the mobile phase.

The developed method produced sharp, symmetrical, and well-resolved peaks with acceptable retention times. Validation studies performed according to ICH guidelines demonstrated satisfactory linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ. Assay results confirmed that the developed method is reliable and suitable for routine quality control analysis of gel formulations.

Overall, the developed RP-HPLC method is simple, reproducible, cost-effective, and suitable for regular pharmaceutical analysis, stability studies, and quality assurance applications in pharmaceutical industries and research laboratories.

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