IMPROVED UV-Vis SPECTROPHOTOMETRIC DETERMINATION OF KETOPROFEN IN TABLETS USING METHANOL AS AN ALTERNATIVE SOLVENT

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Abstract: Ketoprofen is a potent nonsteroidal anti-inflammatory drug (NSAID) belonging to the arylpropionic acid class, extensively used in clinical practice for the management of pain, inflammation, and various rheumatic and musculoskeletal conditions. Its mechanism of action involves the non-selective inhibition of cyclooxygenase enzymes (COX-1 and COX-2), thereby reducing the biosynthesis of prostaglandins responsible for inflammatory processes. Due to its broad therapeutic application and increasing global consumption, precise, reliable, and cost-effective analytical methods are essential to ensure the quality, safety, and efficacy of ketoprofen containing pharmaceutical products. The pharmaceutical industry is constantly evolving and placing greater emphasis on the development and optimization of analytical techniques that are not only accurate and robust but also environmentally sustainable and economically viable. The aim of this research paper is twofold: firstly, to provide a comprehensive review of an existing analytical method for the content determination of ketoprofen in tablets, and secondly, to propose a modified method that utilizes methanol as an alternative solvent to 96% ethanol, with the goal of enhancing analytical efficiency and precision. The experiments in the study were conducted under strictly controlled laboratory conditions using spectrophotometric techniques. The results demonstrated that methanol, due to its superior solvent properties and increased solubility for ketoprofen, facilitated a more efficient extraction of the active pharmaceutical ingredient from the tablet matrix. Consequently, the modified method displayed improved absorbance values and reduced variability across replicates, while maintaining compliance with standard pharmacopeial guidelines. These results suggest that replacing 96% ethanol with methanol may contribute to a more efficient and streamlined workflow in routine pharmaceutical analysis. While methanol offers analytical advantages, it is important to consider the environmental and safety profiles of solvents used in quality control laboratories. Future research may explore greener alternatives or the use of advanced techniques such as solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC) to further enhance the sensitivity and sustainability of ketoprofen analysis. In conclusion, the findings of this study indicate that the proposed method offers measurable improvements in analytical performance and practicality. It is simple, reproducible, and suitable for routine quality control settings, demonstrating improved performance under modified conditions compared to the standard method described in the European Pharmacopoeia. Moreover, this work underscores the importance of continual method optimization in pharmaceutical analysis and provides a solid foundation for further refinement and validation of analytical protocols for ketoprofen analysis.
Keywords: ketoprofen, UV-Vis method, assay

1. INTRODUCTION

Ketoprofen is a nonsteroidal anti-inflammatory drug (NSAID) widely used for the treatment of pain, inflammation, and fever, as well as in managing chronic conditions such as rheumatoid arthritis, osteoarthritis, and dysmenorrhea

(Jamali & Brocks, 1990; Liversidge G.G., 1981). It functions by inhibiting the cyclooxygenase (COX) enzymes involved in prostaglandin synthesis, with a particularly strong effect on COX pathways compared to other NSAIDs (Mariniello et al., 2025). According to the Anatomical Therapeutic Chemical (ATC) classification, ketoprofen is listed under the code M02AA10 (RxReasoner, n.d.).

Ketoprofen is a chiral compound with a single stereocenter, giving rise to two enantiomers that differ in spatial configuration. Despite the pharmacological activity being attributed entirely to the (S)-enantiomer, ketoprofen is routinely administered as a racemic mixture. The (R)-enantiomer, while inactive, does not interfere with the biological function of its active counterpart, thus permitting the use of both forms in standard formulations (Jamali & Brocks, 1990; Liversidge G.G., 1981).

The substance is a faintly colored, crystalline solid, lacking both odor and taste, but potentially irritating in aerosolized form. The compound is characterized by the molecular formula $C_{16}H_{14}O_3$ and molecular weight of 254.09 with an IUPAC name of 2-(3-benzoylphenyl) propanoic acid (Kumar et al., 2024; Ph. Eur. 11, 2023). Structurally, it falls under benzophenones and monocarboxylic oxo acids (National Center for Biotechnology Information, n.d.).

In terms of solubility, ketoprofen is partially soluble in water but dissolves well in organic solvents such as ethanol, methanol, chloroform, ethyl acetate, and acetone. These properties, alongside its stereochemistry and solvent interactions, are critical in the context of pharmaceutical formulation and analysis (Kantor, 1986).

In pharmaceutical quality control, precise and efficient analytical methods are required to ensure that the drug content in formulations falls within the acceptable range of 95-105% of the labeled claim (Lisboa et al., 2022). Among the available techniques for quantifying ketoprofen, UV-Vis spectrophotometry is widely favored in routine analysis due to its simplicity, cost-efficiency, and rapid performance compared to more complex techniques such as HPLC. Validated pharmacopoeial method employs 96% ethanol as the solvent and specifies a wavelength of 255 ± 2 nm for absorption measurement, corresponding to the maximum absorbance of ketoprofen in ethanol/methanol (Gavat, 2023). However, the reliability and sensitivity of spectrophotometric analysis can be significantly influenced by experimental variables, particularly the choice of solvent (Talath & Hani, 2024). Solvent properties such as polarity, transparency at the detection wavelength, and interaction with the analyte can affect solubility, absorbance intensity, and baseline stability (Aravindhan et al., 2023). In some cases, replacing ethanol with a more polar solvent like methanol may improve signal clarity, extraction efficiency, or reduce background interference (Podloucká et al., 2025).

The aim of this study is to optimize the existing spectrophotometric method for ketoprofen content determination by introducing methanol as an alternative to ethanol. The study compares results obtained using the modified method against those of the validated pharmacopoeial procedure in order to assess whether methanol enhances analytical performance. The findings may contribute to improved practices in routine quality control by offering a potentially more efficient and reproducible alternative to the standard method.

2. MATERIALS AND METHODS

The reagents used for the experiments were of analytical grade and suitable for pharmaceutical analysis. All solvents and standards were prepared and handled according to standard laboratory protocols.

The validated method utilized 96% ethanol (Alkaloid AD Skopje), while the optimized method employed methanol (VWR, lot 24K123005) as the extraction and diluting solvent. The reference standard, Ketoprofen certified reference material (CRM) with a purity of 99.8% (batch LRAD1874), was used for calibration. Analytical weighing was conducted on a Mettler Toledo XPR10 Analytical balance, capable of six-decimal precision.

For sample preparation, approximately 20 tablets (average weight 232.40 mg) were finely ground and homogenized. A representative portion equivalent to one tablet was accurately weighed and transferred to a 100 mL volumetric flask. The sample was dissolved in approximately 50 mL of the corresponding solvent, subjected to ultrasonication for 15 minutes, followed by mixing for another 15 minutes, then diluted to volume. The resulting solution was filtered through fine-pore blue filter paper.

Subsequent dilutions were performed in two steps: 5 mL of the filtrate were diluted to 50 mL, followed by 3 mL of that intermediate solution being further diluted to 50 mL, yielding a final working concentration of approximately 0.06 mg/mL. Two replicate samples were prepared for each method.

The analysis was conducted using a Shimadzu UV-1800 spectrophotometer (serial no. A11635372302). A full UV scan was recorded to confirm the identity and λ_{max} of ketoprofen, which was found at 254 nm, consistent with literature values of 255 ± 2 nm. The respective solvent (ethanol or methanol) was used as the blank. Absorbance values were recorded in duplicate for each sample, and results from both methods were compared to assess reproducibility and accuracy.

3. RESULTS AND DISCUSSIONS

To achieve the aim of this study, optimizing the spectrophotometric method for the quantification of ketoprofen in tablets, analyses were first performed using the officially validated pharmacopoeial method. This was followed by a comparative analysis using the same tablet batch, identical sample preparation protocol, and instrumentation, with the only variable being the substitution of 96% ethanol with methanol as the solvent. This controlled setup ensured that any observed differences in the analytical outcome could be attributed solely to the solvent change.

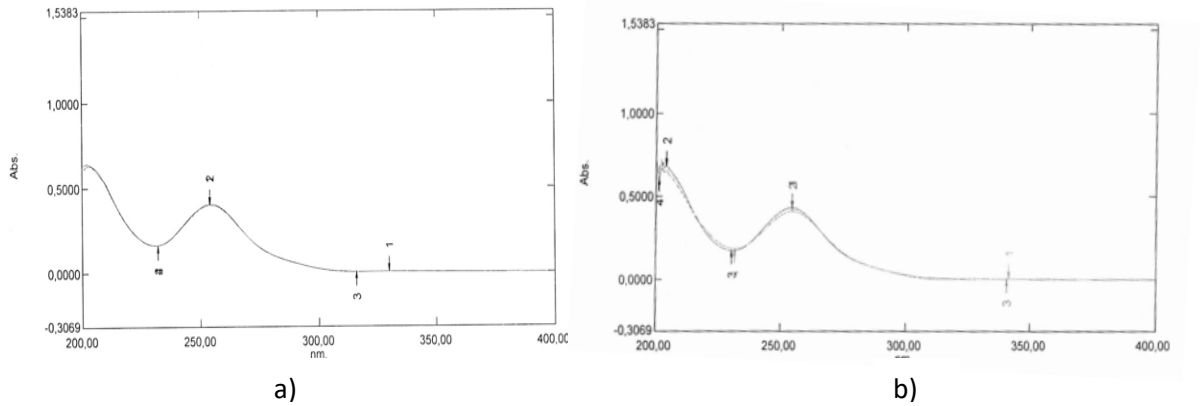
Initially, full UV spectra were recorded for both the validated and optimized methods to confirm the identity of the active pharmaceutical ingredient **(**API) and to assess the wavelength of maximum absorption (λ_{max}). Figure 1a shows the spectrum obtained with the validated method (ethanol), while Figure 1b presents the spectrum using the optimized method (methanol). The overall spectral profiles remained consistent, confirming the structural identity of ketoprofen across both methods and suggesting that solvent substitution does not alter the fundamental chromophoric behavior of the analyte. However, minor spectral differences were observed: a slightly bathochromic shift and a hyperchromic effect were noted when methanol was used.

The bathochromic shift observed, characterized by a subtle red-shift in the absorption maximum when using methanol over ethanol, is well explained by solvent polarity and hydrogen-bonding dynamics. Methanol, which is more polar solvent than ethanol, with greater hydrogen-bond donating ability, stabilizes the excited π-electron system of ketoprofen more effectively than ethanol, thus lowering the energy required for π→π* transitions and shifting λ_{max} toward longer wavelengths. While the overall shift remains minor and does not necessitate changing the analytical wavelength, it provides valuable mechanistic insight into solvent-analyte interactions (Olivares et al., 2023).

The hyperchromic effect, characterized by an increase in absorbance intensity, was more pronounced in the experiments where methanol was used as a solvent. This observation may be attributed to methanol's improved ability to solubilize ketoprofen and maintain it in a monomeric form. In contrast to ethanol, methanol likely reduces the formation of intermolecular aggregates or dimers, which could otherwise diminish absorption efficiency. The monomeric dispersion of ketoprofen molecules enables more effective π→π* electronic transitions, enhancing overall UV absorption. Additionally, methanol's stronger hydrogen bonding may polarize the electron cloud more effectively, increasing the transition dipole moment and signal clarity. While direct evidence for aggregation prevention in methanol is not established for ketoprofen, similar solvent-induced stabilization effects have been described in structurally related α-arylpropionic acids (Moritake et al., 2025), supporting this proposed mechanism.

Taken together, these solvent-specific effects, though subtle, suggest that methanol provides a slightly more favorable analytical environment for UV-Vis quantification of ketoprofen, supporting its suitability as an optimized alternative in routine quality control.

Figure 1. a) Spectrum obtained with the validated method (ethanol), b) Spectrum using the optimized method (methanol)



Source: Author research

Furthermore, quantitative evaluation of the absorbance values for both the standard and test solutions reveals a clear manifestation of the hyperchromic effect when methanol is employed as the solvent. Specifically, the optimized method consistently yields higher absorbance readings, indicating enhanced molar absorptivity. For example, in the case of Standard 1, the validated method resulted in absorbance values of 0.4038 and 0.4043 across two replicates, whereas the optimized method produced higher and identical values of 0.4265 in both measurements. A similar

trend is evident in the test solution analyses, confirming that the increased absorbance is not incidental but a reproducible outcome associated with solvent substitution.

Following the completion of the spectrophotometric analyses and the collection of absorbance values, the subsequent step involves calculating the ketoprofen content in the tablet samples. The calculation is performed using Equation (1), which relates the measured absorbance of the test solution to that of the reference standard:

$$\chi\% = \frac{A \cdot m_s \cdot 3 \cdot 100 \cdot 50 \cdot 50 \cdot \chi}{A_s \cdot 100 \cdot 50 \cdot m \cdot 5 \cdot 3} \quad (1)$$

Where:

$\boldsymbol{\chi\%}$ represents the average content of ketoprofen expressed as a percentage of the labeled claim; $\boldsymbol{A}$ denotes the absorbance of the test solution; $\boldsymbol{m_s}$ is the corrected mass of the standard; $\boldsymbol{\chi}$ is the average mass of a single tablet, $\boldsymbol{A_s}$ refers to the absorbance of the standard solution and $\boldsymbol{m}$ is the accurately weighed mass of the sample.

This calculation ensures that the assay accurately reflects the proportion of the API in the dosage form relative to its declared content.

In Table 1, the detailed absorbance values for all measured samples using both the validated and optimized methods are presented.

Table 1. Detailed absorbance data for all measured samples under both methods

Validated method measurements		**Optimized method measurements**	
Sample	**Absorbance**	**Sample**	**Absorbance**
Standard solution 1/1	0.4038	Standard solution 1/1	0.4265
Standard solution 1/2	0.4043	Standard solution 1/2	0.4265
Standard solution 2/1	0.4120	Standard solution 2/1	0.4259
Standard solution 2/2	0.4121	Standard solution 2/2	0.4261
Test solution 1/1	0.3963	Test solution 1/1	0.4041
Test solution 1/2	0.3964	Test solution 1/2	0.4042
Test solution 2/1	0.3966	Test solution 2/1	0.4042
Test solution 2/2	0.3967	Test solution 2/2	0.4042

Source: Author research

Using Equation (1) and the absorbance values presented in Table 1, the percentage content of ketoprofen in the tablet formulations was calculated. The analysis showed that the average content determined using the validated method was 95.27%, whereas the optimized method yielded a slightly higher average content of 97.43%. Both values fall within the acceptable pharmacopoeial range of 95-105%, validating the applicability of both methods for routine quality control. However, the higher assay value observed with methanol merits deeper interpretation.

At first glance, the result appears contradictory, as the molar solubility of ketoprofen in methanol (1.71 mol/L) is slightly lower than in ethanol (1.85 mol/L) (Filippa et al., 2015). Nevertheless, the outcome cannot be explained by solubility alone. Factors such as solubilization kinetics, matrix effects, solvent-analyte interactions, stability under analytical conditions, and evaporation behavior must be considered. Methanol, being a smaller and more polar molecule, forms stronger hydrogen bonds with ketoprofen and may facilitate faster and more efficient solvation of the API during sample preparation. This contributes to a more homogeneous dispersion of ketoprofen in its monomeric form, enhancing absorbance and signal clarity through improved $\pi \rightarrow \pi^*$ electronic transitions.

Furthermore, the stability of ketoprofen in methanol is generally higher, which may reduce degradation or aggregation during handling and storage. Another contributing factor is solvent evaporation: ethanol exhibits a higher vapor pressure and evaporates more readily than methanol. In open systems or during extended preparation times, this may lead to minor losses of solvent volume, inadvertently increasing solution concentration variability or reducing the actual analyte concentration detected during measurement.

Therefore, although ethanol presents a marginally higher intrinsic solubility for ketoprofen, the practical advantages of methanol, such as enhanced stability, slower evaporation, and reduced interference, translate into better reproducibility and slightly higher detected content, supporting the validity of the optimized method.

4. CONCLUSION

The comparative analysis between the validated method using ethanol and the optimized method using methanol demonstrates that methanol offers clear analytical advantages, particularly in terms of improved absorbance, enhanced signal clarity, and slightly higher detected ketoprofen content. Importantly, all results obtained using

methanol remained within the pharmacopoeial acceptance range (95-105%), confirming the method's suitability for routine pharmaceutical analysis.

These findings suggest that methanol can serve as a viable alternative solvent in the spectrophotometric assay of ketoprofen, with potential to improve method efficiency and analytical reproducibility. Nevertheless, several practical considerations must be addressed before implementation in routine practice. Methanol's significantly higher cost (approximately 1490 MKD per liter compared to 320 MKD for ethanol) may limit its feasibility in resource-constrained settings. Additionally, methanol's toxicity and flammability, particularly its ability to burn with an invisible flame, raise critical concerns for laboratory safety (The OSHA Hazard Communication Standard 29 C F, 2025).

Therefore, while methanol presents analytical benefits, its adoption should be contingent upon proper safety infrastructure, including fume hoods, fire control systems, and trained personnel. If future changes in solvent pricing or regulatory frameworks favor methanol, the method described herein could be fully validated and implemented, contributing to enhanced performance in pharmaceutical quality control.

REFERENCES

Aravindhan, R., Hu, J., & Momeen, M. U. (2023). Role of the solvent polarity on the optical and electronic characteristics of 1-iodoadamantane. *RSC Advances*, *13*(42), 29489–29495. https://doi.org/10.1039/d3ra05297d

Filippa, M. A., Melo, G. M., & Gasull, E. I. (2015). Ketoprofen solubility in organic solvents and aqueous co-solvent systems: Interactions and thermodynamic parameters of solvation. *J Pharm Chem Biol Sci*, *3*(4), 440–453.

Gavat, C.-C. (2023). *Ultraviolet (UV) Spectrophotometric Analysis of Ketoprofen in Tablets–Statistical Validation of Proposed Method*. 60. https://doi.org/10.3390/iocn2023-14442

Jamali, F., & Brocks, D. R. (1990). Clinical Pharmacokinetics of Ketoprofen and Its Enantiomers. *Clinical Pharmacokinetics*, *19*(3), 197–217. https://doi.org/10.2165/00003088-199019030-00004

Kantor, T. G. (1986). Ketoprofen: A Review of Its Pharmacologic and Clinical Properties. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *6*(3), 93–102. https://doi.org/10.1002/j.1875-9114.1986.tb03459.x

Kumar, B., Sahani, V., & Patil, S. (2024). Review on Ketoprofen (Anti-Inflammatory Drug). *Journal for Research in Applied Sciences and Biotechnology*, *3*(6), 41–50. https://doi.org/10.55544/jrasb.3.6.6

Lisboa, U. De, Caroline, V., & Reichert, D. (2022). *Analytical Methods Validation in the Regulatory Framework. Experimental Designs, Optimization, And Evaluation: The Case of Stability Testing - ProQuest*. https://www.proquest.com/docview/3161879192? Theses&fromopenview=true&pq-origsite=gscholar&sourcetype=Dissertations

Liversidge G.G. (1981). Ketoprofen. *Analytical Profiles of Drug Substances and Excipients*, *10*, 443–471. https://doi.org/10.1016/S0099-5428(08)60647-4

Mariniello, D. F., Pagliaro, R., D'Agnano, V., Schiattarella, A., Perrotta, F., & Bianco, A. (2025). Ketoprofen Lysine Salt Versus Corticosteroids in Early Outpatient Management of Mild and Moderate COVID-19: A Retrospective Study. *Pharmacy*, *13*(3), 65. https://doi.org/10.3390/pharmacy13030065

Moritake, K., Tsuchida, T., Koga, R., Hasegawa, K., Kuwashima, W., Kataoka, H., Goto, S., & Terada, H. (2025). Equilibrium of monomers, dimers, and polymeric aggregates in the α-aryl-propionic acid-type analgesics naproxen, ketoprofen, and ibuprofen: Comparative study with oxicam-type meloxicam and piroxicam. *International Journal of Pharmaceutics*, *670*. https://doi.org/https://doi.org/10.1016/j.ijpharm.2025.125167

National Center for Biotechnology Information. (n.d.). *PubChem Compound Summary for CID 3825, Ketoprofen*. Retrieved July 31, 2025, from https://pubchem.ncbi.nlm.nih.gov/compound/R_-Ketoprofen

Olivares, F. G., Lazcano, F. G., Castillo, M. O., Barúa, M. G., Escalada, J. P., & Vitorino, G. P. (2023). Solvatochromism and Thermochromism of Fluconazole: An Experimental and Theoretical Study. *Journal of the Brazilian Chemical Society*, *34*(10), 1380–1397. https://doi.org/10.21577/0103-5053.20230050

Podloucká, P., Polišenská, I., & Jirsa, O. (2025). Effect of the extraction solvent and method on the determination of the total polyphenol content in different common buckwheat (Fagopyrum esculentum Moench) varieties. *Food and Nutrition Research*, *69*(5). https://doi.org/10.29219/fnr.v69.9834

RxReasoner. (n.d.). *ATC Group: M02AA10 Ketoprofen*. Retrieved July 28, 2025, from https://www.rxreasoner.com/atccodes/M02AA10

Talath, S., & Hani, U. (2024). Spectrophotometric Methods in Pharmaceutical Analysis: Principles, Reagents, and Applications. *International Journal of Environmental Sciences & Natural Resources*, *34*(3). https://doi.org/10.19080/ijesnr.2024.34.556391

The OSHA Hazard Communication Standard 29 C F. (2025). Safety Data Sheet. *Material Safety Data Sheet*, *4*(2), 1–6. https://valenzgroup.com/wp-content/uploads/2025/04/Valenz-Methanol-US-en-SDS_4-7-25.pdf?utm