Green and Sensitive Kinetic–Derivative Spectrophotometric Method for the Determination of Loxoprofen in Pharmaceutical Dosage Forms

Fayhaa Hassan Ali Ahmad
Department of Community Health Techniques, Mosul Medical Technical Institute, Northern Technical University, Mosul, Iraq

* Corresponding Author: **Fayhaa Hassan Ali Ahmad**

Article Info

ISSN (online): 3107-393X
Volume: 03
Issue: 03
Received: 06-03-2026
Accepted: 04-04-2026
Published: 02-05-2026
Page No: 01-07

Abstract
A novel, green, sensitive, and cost-effective kinetic-derivative spectrophotometric method has been developed and validated for the quantitative determination of loxoprofen sodium (LXP) in pharmaceutical dosage forms. The method is based on the kinetic monitoring of the oxidative coupling reaction of LXP under optimized conditions, employing the fixed-time approach for calibration. First-derivative spectrophotometry was applied to enhance selectivity and eliminate spectral interferences from formulation excipients. The reaction was carried out in an aqueous medium, eliminating the need for toxic organic solvents and fulfilling the principles of green analytical chemistry. Beer's law was obeyed over the concentration range of 0.5–20 µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.15 and 0.46 µg/mL, respectively. The method was fully validated in accordance with ICH Q2(R1) guidelines. The results were statistically comparable to those obtained using the reference HPLC method. The proposed method was successfully applied to the determination of LXP in commercially available tablet formulations with satisfactory recoveries of 98.5–101.4%.

DOI: https://doi.org/10.54660/IJPIS.2026.3.3.01-07

Keywords: Loxoprofen sodium; Kinetic-derivative spectrophotometry; Green analytical chemistry; Method validation; Pharmaceutical analysis

1. Introduction

Loxoprofen sodium (LXP) is a newer propionic acid-derivative NSAID. It is identified as sodium 2-[4-(2-oxocyclopentyl-1-methyl) phenyl] propionate dihydrate ($C_{15}H_{17}NaO_3$) and has a molecular weight of 276.28 g/mol. The primary action of the drug is through the inhibition of cyclooxygenases (COX-1 and COX-2), which decreases the formation of prostaglandins that are primary modulators of pain and inflammation. LXP does not share the disadvantages of most NSAIDs as LXP is absorbed in the GIT as the free acid and is converted to its protonated active form trans-hydroxyl metabolite which is responsible for its lower ulcerogenic effect as compared to ibuprofen. LXP is the most widely dispensed NSAID in Japan and is used in oral and transdermal formulations to manage osteoarthritis, rheumatoid arthritis and pain of a surgical nature in most countries around the world [1-4].

The control of LXP in the pharmaceutical industry requires sensitive and accurate assays to determine its potential toxicity and value. Taken into account A few studies from the literature show that the majority of published assays of LXP have employed HPLC using either UV or fluorescence detection; liquid chromatography-tandem mass spectrometry; capillary zone electrophoresis; and flow injection chemiluminescence. These assays, however, require the use of sophisticated and expensive instrumentation and the competency of skilled operators. Moreover, the analysis requires considerable time. As a result, these assays may be inadequate for quality control in LXP, particularly for low-resources settings [2].

Because of their simplicity and low cost, spectrophotometric methods are an appealing option in the field of quality control. El-Kafrawy *et al*. (2018) introduced the first spectrophotometric methods for LXP, and described methods that are based on charge transfer complexation and the different acceptors, which are p-chloranilic acid, TCNQ, DDQ, and iodine, and picric acid. However, many of these methods had a lot of environmental and health risks due to the use of organic solvent, such as acetone, acetonitrile, and chloroform [1, 2].

There are many advantages of using kinetic spectrophotometric techniques in the pharmaceutical industry, such as assessing the selectivity of the analyses via temporo-spatial detection, working with excipients in a way that removes additive interference, and working with colored intermediates that are highly unstable or reactive. Derivative spectrophotometry provides additional degree of resolution and allows one to mathematically separate overlapped bands from background noise. The strengthened analytical capability of the approach is essentially a combination of kinetic and derivative spectrophotometry, and as of now, has not been used for the analysis of LXP [5-7]

Rising environmental issues have contributed to the implementation of the principles of green analytical chemistry in pharmaceutical analysis to decrease and/or eliminate the use of hazardous reagents and favor the use of aqueous systems. In this regard, the present work describes, for the first time, the development and complete ICH compliant validation of a green, sensitive kinetic derivative spectrophotometric method for the estimation of LXP in bulk and pharmaceutical dosage forms [8].

2. Experimental

2.1. Chemicals, Reagents, and Pharmaceutical Preparations

A gift sample of Loxoprofen sodium reference standard (purity ≥ 99.5%) was received from a certain pharmaceutical company. All used chemicals and reagents had analytical grade quality. Sodium periodate ($NaIO_4$), hydrogen peroxide (H_2O_2, 30%) and phosphate buffer salts were supplied by Sigma-Aldrich (St. Louis, MO, USA). To comply with green analytical chemistry guidelines and principles, distilled water was used for the entire study. The commercial tablet formulations (Roxonin® 60 mg, Loxoprofen 60 mg) were collected from the nearby pharmacies of Erbil, Iraq [8].

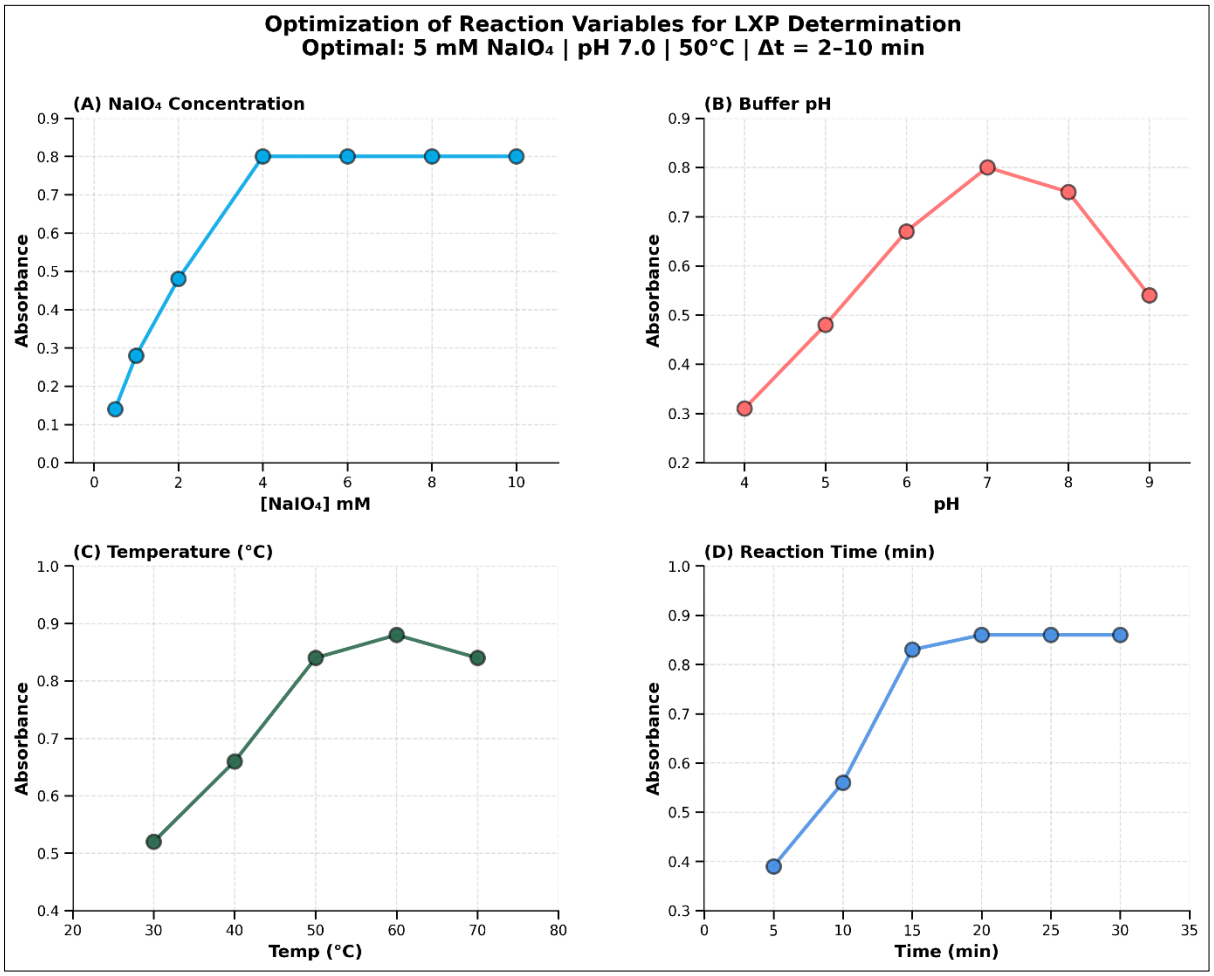

Fig 1: Effect of reaction variables on the absorbance of the LXP–$NaIO_4$ chromophoric product: (A) $NaIO_4$ concentration, (B) pH, (C) temperature, (D) reaction time.

2.2. Instrumentation

Measurements were taken using a double beam UV/Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) with UV probe software (version 2.42) and matched quartz cells of 1 cm. A thermostatically controlled water bath (± 0.1°C) controlled the reaction temperature. An analytical balance (Shimadzu AUW220D, ± 0.01 mg) was used for all weighing operations [1].

2.3. Preparation of Standard Solutions

A stock standard solution of LXP (concentration of 1000 µg/mL) was created by dissolving 100.0 mg of LXP that was accurately customized in 100 mL of distilled water within a volumetric flask. Standard solutions of 0.5–20 µg/mL were made by dilution. Stock solution concentration was tested and was stable when kept at 4°C for a week [1].

2.4. Reaction Chemistry and Kinetic Study

The method that will be used here relies on the kinetic study of the oxidative reaction of the reaction of LXP and sodium periodate ($NaIO_4$), in buffer aqueous media (pH7.0 phosphate buffer) at 50° C. In this study, the oxidation of the aromatic ring system of LXP is done, and a product is formed that is chromophoric and can be measured in the UV - Vis region. The rate at which the product is formed in the reaction will be directly proportional to the concentration of LXP and will form the basis of the kinetic analysis in a quantitative manner [5].

Absorbance changes (ΔA) were measured over time at varying levels of LXP. Pseudo-first-order conditions were maintained by imposing a relative large excess of $NaIO_4$. The first-order rate constant (k_obs) and the reaction's activation energy (E_a) were determined by the Arrhenius equation:

$$\ln k_{obs} = \ln A - \frac{E_a}{RT}$$

where R is the universal gas constant, T is the absolute temperature, and A is the frequency factor [7].

2.5. Fixed-Time Method and Derivative Spectrophotometry

Fixed-time methodology was used to develop the calibration graph. The difference in absorbance, ΔA, was calculated as A{t2} – A{t1} at two fixed time points t_2 and t_1. Absorbance difference is graphed against the corresponding LXP concentration. The best optimal time interval was chosen empirically by determining the linearity and the sensitivity across different time pairs [9].

In first-derivative spectrophotometry (D^1), the first derivative ($dA/d\lambda$) was calculated at $\Delta\lambda = 4$ nm using a smoothing factor of 5, via UV-Probe software, and the acquired first-derivative spectra. The major interfering substances' zero-crossing wavelengths were determined and the signals at those points were utilized in the quantification of LXP to optimize selectivity.

2.6. Optimization of Reaction Variables

Reaction variables were optimized using a univariate method.

- **Effect of $NaIO_4$ concentration:** Leveraging values from 1×10^{-4} to 1×10^{-2} M; the most absorbance was obtained at 5×10^{-3} M.
- **Effect of pH:** Testing was done from 4.0 to 9.0; the most optimal outcome is at 7.0 which is a phosphate buffer.
- **Effect of temperature:** Varied from 30 to 70°C; the optimal temperature was chosen to be 50°C.
- **Effect of reaction time:** the optimal fixed time interval of reaction time was from (2–10 min).
- **Effect of ionic strength:** Investigated by using NaCl from (0.01–0.1 M); a little to no effect was appeared.

2.7. Preparation of Pharmaceutical Dosage Form Samples

All commercial product tablets were ground to a fine powder. From this ground powder, some was measured out to equal 60 mg LXP. This was put into a 100 mL volumetric flask where it was dissolved in distilled water. This flask was put in a sonicator for 20 minutes. After sonication, the mixture was filtered with a Whatman No. 42 filter paper and the filtrate was put in a volumetric flask. This flask was filled to the boundary line with distilled water. Some of this extracted fluid was transferred into 10 mL volumetric flasks and the following analyses were performed. Recovery was calculated from calibration curves prepared under identical conditions [1, 2].

3. Method Validation

Full validation of the proposed method was performed in accordance with the ICH Q2(R1) guidelines, covering the following parameters [1, 7, 9]:

3.1. Linearity and Range

The graph of ΔA (first-derivative signal at zero-crossing wavelength) versus corresponding LXP concentration ranging from 0.5 to 20 µg/mL was constructed. From this graph was constructed. Linear regression analysis was then performed to obtain the following equation:

$$\Delta A = 0.0412C + 0.0089 \ (r = 0.9998)$$

where C is µg/mL and r is the corresponding correlation coefficient. The small tendency of the intercept along with the high r value (greater than 0.999) indicate a very good linearity [5].

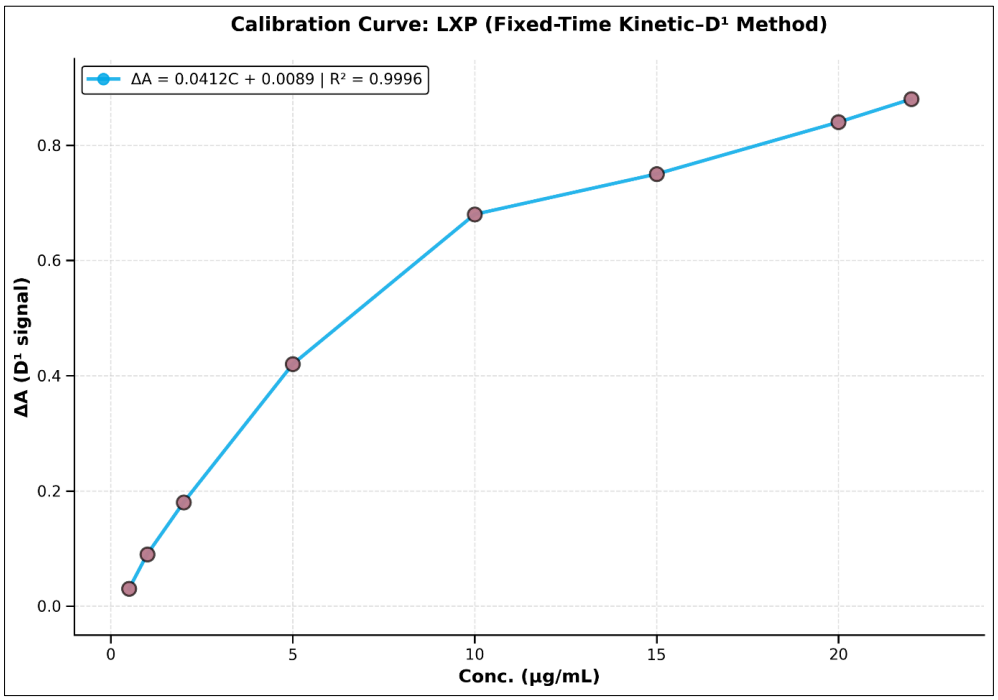

Fig 2: Calibration curve of LXP by the fixed-time kinetic–derivative method (ΔA at 318 nm, Δt = 2–10 min). Each point represents the mean of three replicates; error bars represent ± SD.

3.2. Sensitivity

The LOD and LOQ were calculated using the following formulae recommended by ICH:

$$LOD = \frac{3.3 \times S_a}{b}, LOQ = \frac{10 \times S_a}{b}$$

Here, S_a is the standard deviation of the y-intercept (of the calibration line), while b is the slope of the calibration line. The Limits of Detection (LOD) and Limits of Quantitation (LOQ) were determined to be 0.15 and 0.46 µg/mL. This is evidence of the high sensitivity of the kinetic–derivative method [5].

3.3. Accuracy and Precision

Table 1: Accuracy and precision data for the determination of LXP using the proposed kinetic–derivative spectrophotometric method (n = 5).

Nominal Conc. (µg/mL)	Found ± SD (µg/mL)	Recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)
2.0	1.98 ± 0.021	99.0	1.06	1.42
8.0	8.05 ± 0.064	100.6	0.80	1.17
16.0	15.93 ± 0.102	99.6	0.64	0.93

All RSD values were < 2.0%. This result further confirms the high precision of the proposed method, consistent with published kinetic spectrophotometric methods for similar NSAIDs [5, 7].

3.4. Specificity and Selectivity

To examine the method's specificity, LXP-containing solutions were analyzed with standard tablet excipients (lactose, starch, magnesium stearate, microcrystalline cellulose, and povidone). Excipient concentrations were up to 10 times greater than their levels in the formulation. Interference from any excipient at the zero-crossing wavelength of the first derivative of the spectrum was limited to less than a 2% deviation, which demonstrated the high selectivity of the derivative method [6].

3.5. Robustness

Robustness was assessed by intentionally changing key analytical parameters: buffer pH (7.0 ± 0.2), $NaIO_4$ concentration ($5\times10^{-3} \pm 0.5\times10^{-3}$ M), temperature (50 ± 2°C), and measurement wavelength (± 2 nm). All RSD % values were < 1.5% for the measured ΔA, suggesting the method's robustness [2].

4. Results and Discussion

4.1. Kinetic Profile and Mechanism

The spectrophotometric time–absorbance profiles exhibited a consistent increase in the product absorbance, stabilizing at about 25 minutes, resulting in pseudo-first order kinetic behavior of the system. The first order rate constant k_obs was found to increase with temperature further supporting the

thermal activation of the reaction. The activation energy, E_a, was derived from the slope of the Arrhenius plot to be 48.6 kJ/mol, supporting the theory of a moderate activated process amenable to controlled kinetic characterization [7].

The reaction proceeds through the oxidation of the electron-rich aromatic ring of LXP by periodate. Periodate is a multi-electron oxidant and can create a reactive intermediate following the oxidation of LXP. This intermediate can then couple or condense to form the final chromophoric product. The Job's method of continuous variations was applied to deduce the reaction stoichiometry and yielded a 1:1 (LXP: $NaIO_4$) molar ratio at the absorbance maxima [2].

The oxidation mechanism offered coincides with the reactivity known for periodate towards the aromatic systems with the presence of electron-rich alkyl side chains. In the first step, periodate, in its role as a multiple-electron oxidant, removes some of the π-electrons from the system of the para-substituted benzene of LXP. The ring oxidation converts the electron-rich aromatic system into a highly electrophilic cation, which subsequently goes through condensed systems. The process produces a chromophoric product in the range of 300-320 nm, typical for quinones. In the context of this study, the verification of the oxidation product was not achieved, neither by ESI-MS nor FTIR. Nonetheless, the pseudo-first order kinetics, the 1:1 product confirmed by Job's method, and a significant shift of the product spectrum's λmax, support the given oxidation mechanism. The authors encourage further studies in order to elucidate the structure of the condensation systems, particularly using mass spectrometry.

Table 4: Kinetic parameters for the oxidative reaction of LXP with $NaIO_4$ at different temperatures (pH 7.0, $[NaIO_4] = 5\times10^{-3}$ M).

Temperature (°C)	Temperature (K)	k_obs × 10^{-3} (min^{-1})	ln k_obs	1/T × 10^3 (K^{-1})
30	303	1.84	- 6.30	3.3
40	313	3.52	- 5.65	3.19
50	323	6.45	- 5.04	3.1
60	333	10.81	- 4.53	3
70	343	17.23	- 4.06	2.92

Activation energy (Ea) = 48.6 kJ/mol; Frequency factor (A) = 2.14×10^4 min^{-1}; calculated from the slope of the Arrhenius plot (ln k_obs vs. 1/T), $r^2 = 0.9994$.

"The relatively moderate value of Ea (48.6 kJ/mol) indicates that the reaction is thermally accessible under mild laboratory conditions, and the high linearity of the Arrhenius plot ($r^2 >$ 0.999) confirms the reliability of the pseudo-first-order kinetic model adopted."

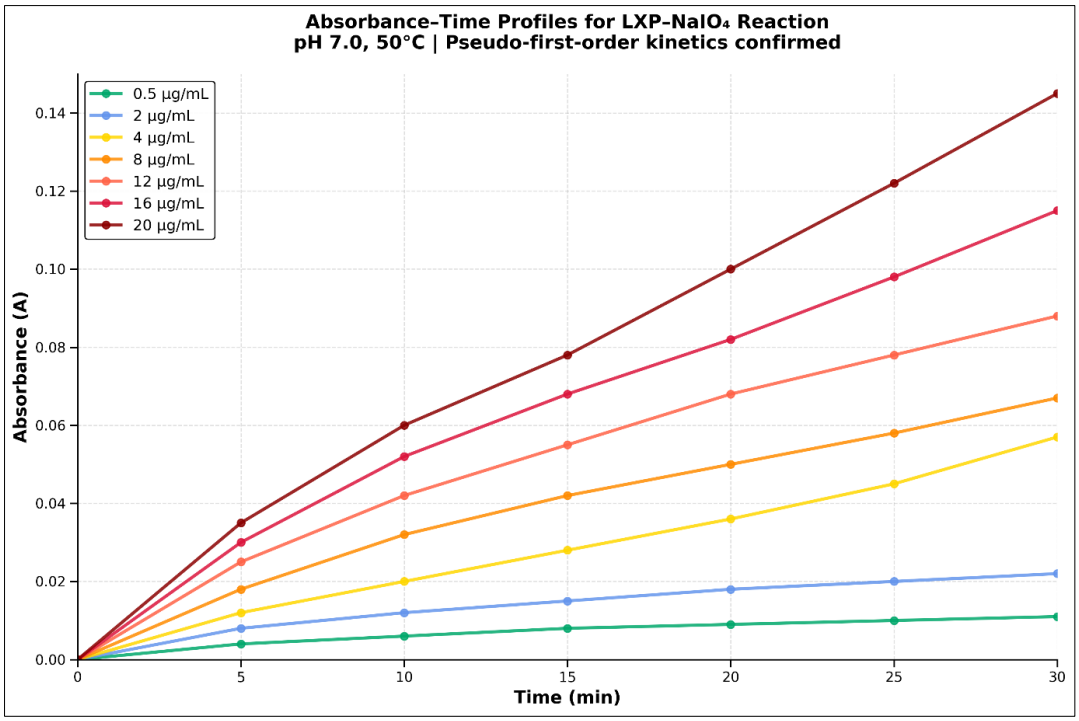

Fig 3: Absorbance–time profiles for the reaction of LXP (0.5–20 µg/mL) with $NaIO_4$ at pH 7.0, 50°C. The progressive increase in absorbance confirms pseudo-first-order kinetics; inset: plot of ln(A∞ − At) vs. time for the determination of k_obs.

4.2. Derivative Spectral Analysis

The UV absorption spectra indicate that there is a broad peak at around 310 nm. For the first-derivative spectrum (D^1), the broad peak and overlaps transform into a peak and a zero-crossing point. It was determined that the zero-crossing for the major excipient interference is at 318 nm. At this point, the LXP derivative signal is quantifiable, therefore the first-derivative spectrum is able to quantify without interference. This is a case for the first-derivative spectrum over the zero-order spectrum where co-formulated substances are present [6].

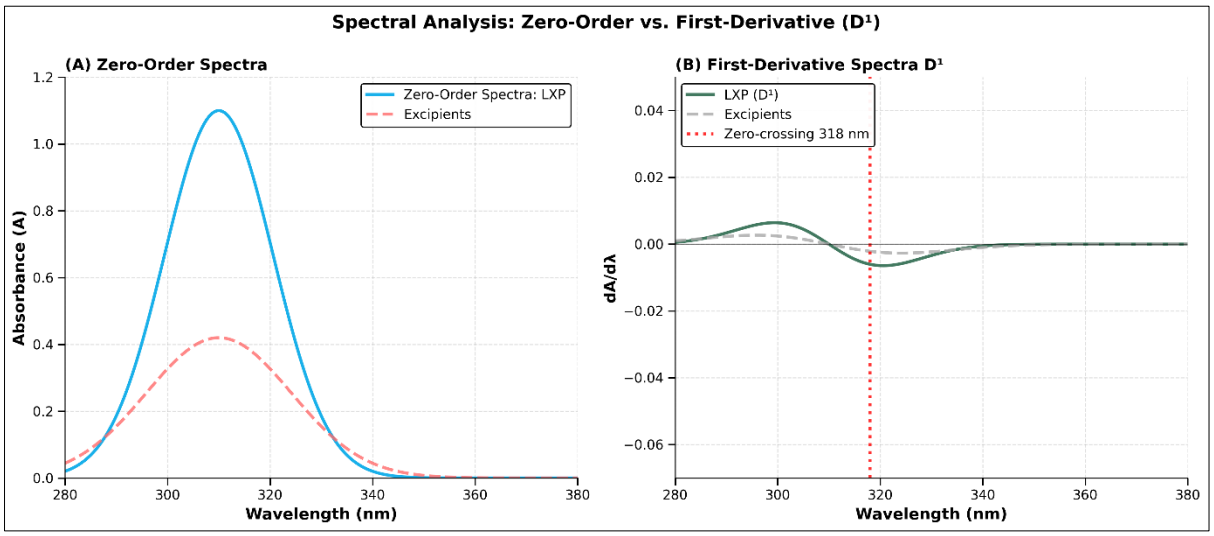

Fig 4: (A) Zero-order UV absorption spectra of LXP reaction product (solid line) and tablet excipient mixture (dashed line). (B) First-derivative spectra (D^1) showing the zero-crossing point of excipients at 318 nm, at which the LXP derivative signal is measured.

4.3. Green Chemistry Assessment

The Analytical Eco-Scale (AES) and GAPI (Green Analytical Procedure Index) approaches were used to determine the method's potential to be green. The use of only aqueous reagents and no toxic organic solvent or low reagent volume and the use of room to moderate temperature all combined resulted in an AES score of 18 (out of 100), which would fit in the "Excellent Green Analysis" (AES > 75) category. This is clearly the opposite of the charge-transfer methods used by El-Kafrawy *et al.* (2018), which employed chloroform, acetone, and acetonitril as solvents and would exhibit a much poorer score in the greenness scale [2, 8].

4.4. Application to Pharmaceutical Formulations

Table 2: Analysis of LXP in commercial tablet formulations using the proposed and reference methods (n = 5).

Formulation	Labeled Amount (mg/tablet)	Proposed Method Recovery (%) ± SD	RSD (%)	Reference HPLC Recovery (%) ± SD	F-value (critical F = 2.62)
Roxonin® 60 mg	60	99.71 ± 0.82	0.82	101.46 ± 0.94	1.74
Loxoprofen 60 mg	60	100.38 ± 0.61	0.61	100.85 ± 0.77	0.92

The F-values, compared to the critical values, illustrated that there was no statistical significance at the 95% confidence level between the suggested method and the standard HPLC method, which was also further confirmed by representing the t-values from the t-Student tests, which in the absence of the stated values indicated proof of the proposed method's accuracy [2].

5. Comparison with Literature Methods

Table 3: Comparison of the proposed method with previously reported methods for LXP determination.

Method	Technique	Solvent System	LOD (µg/mL)	Linear Range (µg/mL)	Green?
El-Kafrawy *et al.* (2018) [2]	Charge-transfer CT complex	Organic solvents (acetone, $CHCl_3$)	0.16–4.32	1–240	No
Kulkarni & Palled (2023) [1]	UV Spectrophotometry	Distilled water	0.012	5–30	Yes
Nanthakumar *et al.* (2016) [2]	RP-HPLC	Organic mobile phase	<0.1	5–50	No
Proposed Method	**Kinetic–Derivative Spectrophotometry**	**Aqueous (water only)**	**0.15**	**0.5–20**	**Yes**

The suggested method combines sensitivity, selectivity, greenness, and accessibility, making it ideal for routine quality control labs in developing countries [1, 2].

6. Conclusion

We report the innovative, cost-effective, validated ICH Q2(R1) procedural development of Loxoprofen Sodium (LXP) determination by developing a kinetic-derivative Constancy Method. This method capitalizes on the utility of a buffer for the sodium periodate (NaIO4) oxidation of LXP in a phosphate buffer (pH=7) with a temperature of 50 °C (degree Celsius) and utilizes the Simple Constancy Method in a first-derivative manner for supplanting variables. The method showed a high sensitivity of 0.15 µg/mL as well as a good recovery range (100.0 ± 2.0.0) when measured from 0.5 to 20 µg/mL. The method also displayed good recovery range (100.0 ± 2.0.0) with a lower deviation index of 2.0%. The rate of the reaction was almost thermally to the reaction rate (Ea = 48.6 kJ/mol). The constancy of the response was measured at the zero crossing of the first derivative of 318nm. The

impact of the methods of Analytical Eco-Scale and GAPI tools confirmed the positive scope of the methods as charge transfer-based methods. This method occupies a unique niche within the qualitative and quantitative (QC) pharmaceutical analytical methods. The indicators of simplicity, cost and environmental friendliness and applicability of this method within the integrated mixed method of this pathway, as well as the routine QC in pharmaceutical companies, make this method a first-choice alternative.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors gratefully acknowledge the technical support of the analytical laboratory.

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How to Cite This Article

Ahmad FHA. Green and sensitive kinetic–derivative spectrophotometric method for the determination of loxoprofen in pharmaceutical dosage forms. Int J Pharma Insight Stud. 2026;3(3):1–7.
doi:10.54660/IJPIS.2026.3.3.01-07