DEVELOPMENT AND VALIDATION OF A SIMULTANEOUS HPLC METHOD FOR QUANTIFICATION OF KETOPROFEN AND METHYLPARABEN IN TOPICAL GEL

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Abstract: *This study has developed a method for simultaneous quantification of ketoprofen and methylparaben in topical gel by reversed-phase HPLC. The method was performed on a Phenomenex C18 column (4.6 mm × 250 mm; 5 µm), using a mobile phase of acetonitrile – 0.1% ortho phosphoric acid (50 : 50, v/v) under isocratic conditions, at a flow rate of 1.0 mL/min, column temperature 35°C, UV detection at 254 nm, and injection volume of 10 µL. The method was validated for specificity, system suitability, linearity, accuracy, precision (repeatability and intermediate precision) in accordance with ICH Q2(R2) guidelines and AOAC 2016. Validation results demonstrated that the method is suitable for simultaneous determination of ketoprofen and methylparaben in topical gel and can be applied in quality control laboratories.*

Keywords: *Simultaneous quantification, ketoprofen, methylparaben, topical gel, HPLC.*

1. INTRODUCTION

In the management of dermatological conditions and soft tissue disorders, topical gel formulations have gained increasing clinical preference due to their favorable drug release profiles, aesthetic acceptability, and patient compliance [1]. The polymeric network structure of gels facilitates active pharmaceutical ingredient (API) stabilization and enhances trans-epidermal penetration [2]. Among non-steroidal anti-inflammatory drugs (NSAIDs), ketoprofen is a potent topical analgesic and anti-inflammatory agent that acts through non-selective inhibition of COX-1 and COX-2 enzymes, and is considered among the most potent COX inhibitors currently available [3, 4].

In addition to the principal API, methylparaben is one of the most widely employed antimicrobial preservatives in semi-solid pharmaceutical preparations, owing to its broad-spectrum antimicrobial activity and well-established safety profile [5]. The simultaneous quantification of ketoprofen and methylparaben within a single analytical procedure not only improves efficiency in terms of time and analytical resources, but also ensures comprehensive quality control of the finished dosage form in accordance with current pharmacopoeia standards [6].

To date, neither the Vietnamese Pharmacopoeia 5th Edition nor major reference pharmacopoeias include a dedicated monograph for the simultaneous assay of these two substances in topical gel preparations. Published methods have either employed complex chromatographic conditions or addressed each analyte independently [7, 8]. Accordingly, this study was undertaken to develop and validate an HPLC method for the simultaneous quantification of ketoprofen and methylparaben in topical gel, with validation conducted in accordance with ICH Q2(R2) [9] and AOAC 2016 [10].

2. MATERIALS AND METHODS

2.1. Equipment, Reagents, and Reference Standards

2.1.1. Equipment and Instrumentation

Agilent HPLC system equipped with a PDA detector; Mettler Toledo pH meter; Mettler MS105 analytical balance (readability: 0.01 mg); Mettler MS3002TS technical balance; ultrasonic bath; micropipettes (1000 µL and 5000 µL); Class A volumetric glassware. All instruments were calibrated and complied with ISO/IEC 17025 and GLP requirements.

2.1.2. Reagents and Reference Standards

Reference standards: Ketoprofen ($C_{16}H_{14}O_3$), assay 100.0%, Lot No. 0105178; Methylparaben ($C_8H_8O_3$), assay 99.5%, Lot No. C0523108.

Solvents and reagents: Acetonitrile HPLC-grade (Merck, Germany); Phosphoric acid 85% (Merck, Germany); Reverse osmosis (RO) purified water.

2.2. Study Design and Methodology

2.2.1. Test Samples

Topical gel samples containing ketoprofen approximately 2.5% (w/w) and methylparaben

0.4% (w/w). Excipients included carbomer, ethanol, triethanolamine, propylene glycol, and purified water. Placebo formulations were prepared identically to the test samples but without the analytes: Placebo 1 (without ketoprofen and methylparaben); Placebo 2 (without methylparaben); Placebo 3 (without ketoprofen).

2.2.2. Methodology

2.2.2.1. Sample Preparation

Diluent (DL): Acetonitrile – 0.1% orthophosphoric acid (50 : 50, v/v).

Ketoprofen stock standard solution (0.25 mg/mL): Accurately weigh about 25 mg of ketoprofen reference standard into a 100 mL volumetric flask; dissolve and dilute to volume with DL.

Methylparaben stock standard solution (0.04 mg/mL): Accurately weigh about 20 mg of methylparaben reference standard into a 50 mL volumetric flask; dissolve and dilute to volume with DL. Transfer exactly 5.0 mL of this solution into a second 50 mL volumetric flask and dilute to volume with DL.

Mixed working standard solution: Transfer exactly 1.0 mL of the ketoprofen stock standard solution and 1.0 mL of the methylparaben stock standard solution into a 10 mL volumetric flask; dilute to volume with DL. The resulting solution contains ketoprofen at 25 µg/mL and methylparaben at 4 µg/mL.

Test solution: Accurately weigh approximately 1 g of gel sample into a beaker. Add approximately 50 mL of DL and sonicate for 15 minutes. Transfer quantitatively into a 100 mL volumetric flask, rinsing the beaker three times and dilute to volume with DL. Pipette exactly 1000 µL into a 10 mL volumetric flask and dilute to volume with DL. Filter through a 0.45 µm membrane filter prior to HPLC injection.

2.2.2.2. Chromatographic Conditions

C18 column (4.6 mm × 250 mm, 5 µm); mobile phase: acetonitrile – 0.1% orthophosphoric acid; mobile phase ratio, flow rate, column temperature (35°C), and detection wavelength (254 nm) were selected based on optimization studies.

3. RESULTS

3.1. Selection of Chromatographic Conditions

3.1.1. Selection of Detection Wavelength

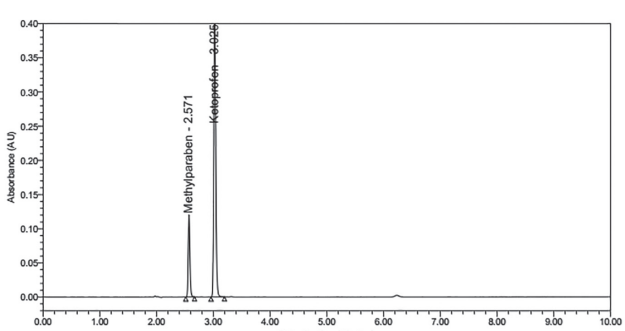
UV absorption spectra of individual standard solutions of each analyte were recorded across the wavelength range 200 – 400 nm. Both ketoprofen and methylparaben exhibited maximum absorption at approximately 254 – 255 nm. Accordingly, 254 nm was selected as the detection wavelength for simultaneous quantification of both compounds.

3.1.2. Selection of Diluent

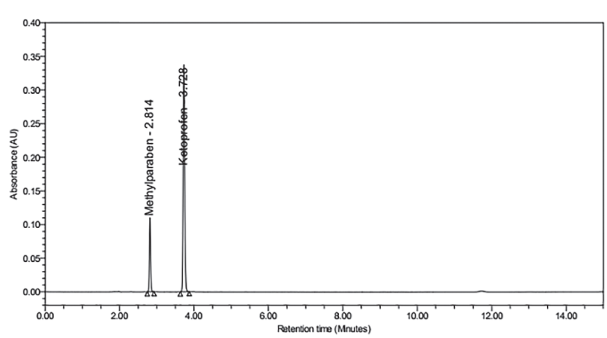
Four solvent systems were evaluated: (1) ACN – 0.1% phosphoric acid (50 : 50, v/v); (2) methanol; (3) absolute ethanol; (4) 90% ethanol. Systems (2) and (4) produced irregular peak shapes and co-eluting interferences. Systems (1) and (3) yielded well-defined, symmetrical peaks with stable retention times; system (1) was preferred due to superior analyte extraction efficiency and higher peak purity. Conclusion: Diluent (1) – acetonitrile – 0.1% phosphoric acid (50 : 50, v/v) – was selected.

3.1.3. Selection of Mobile Phase Ratio

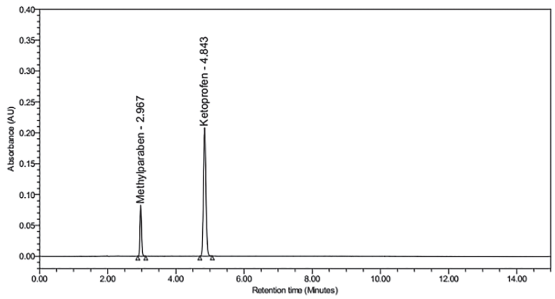
Based on the pKa values of ketoprofen (4.5) and methylparaben (8.4), a mobile phase pH below 4.0 was selected to maintain both analytes in their unionized forms. Four ACN : PA ratios were evaluated (60 : 40; 40 : 60; 50 : 50; 70 : 30). Results are presented in Figure 1 and Table 1.



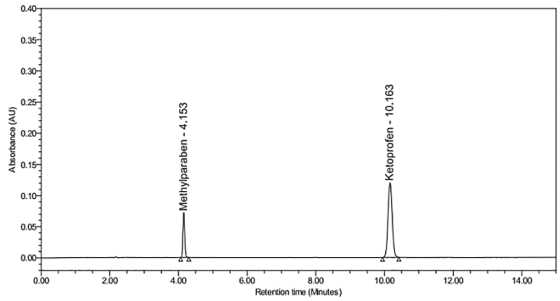
(A) Ratio 70 : 30



(B) Ratio 60 : 40



(C) Ratio 50 : 50



(D) Ratio 40 : 60

Figure 1. *Representative chromatograms obtained under different mobile phase conditions*

Table 1. *Results of mobile phase ratio screening*

ACN : PA Ratio	t_R methylparaben (min)	t_R ketoprofen (min)	Remarks
40 : 60	4.153	10.163	Excessively long analysis time; peak tailing observed
50 : 50	2.967	4.843	Optimal: complete resolution, short analysis time
60 : 40	2.814	3.728	Narrowed peak-to-peak spacing
70 : 30	2.571	3.025	Peaks nearly co-eluted; risk of peak overlap

The 50 : 50 (ACN : PA) ratio was selected as it provided complete baseline resolution of both analytes, a short analysis time (approximately 6 – 7 minutes), and a stable baseline.

3.1.4. Selection of Column Temperature and Flow Rate

Column temperature was evaluated at five levels: 25, 35, 40, 45, and 50°C. At all temperature levels, methylparaben consistently eluted prior to ketoprofen with a stable retention time difference of approximately 2 minutes; peak areas showed minimal variation. A column temperature of 35°C was selected to ensure column longevity and compatibility with standard laboratory operating conditions.

Five flow rates were evaluated: 0.5, 0.8, 1.0, 1.2, and 1.5 mL/min. A flow rate of 1.0 mL/min was selected as it ensured complete analyte separation, an acceptable analysis time, and system backpressure within acceptable limits.

Final chromatographic conditions: Phenomenex C18 column (4.6 mm × 250 mm, 5 µm); mobile phase: ACN – 0.1% orthophosphoric acid (50 : 50, v/v); flow rate: 1.0 mL/min; column temperature: 35°C; detection wavelength: 254 nm; injection volume: 10 µL.

3.2. Method Validation

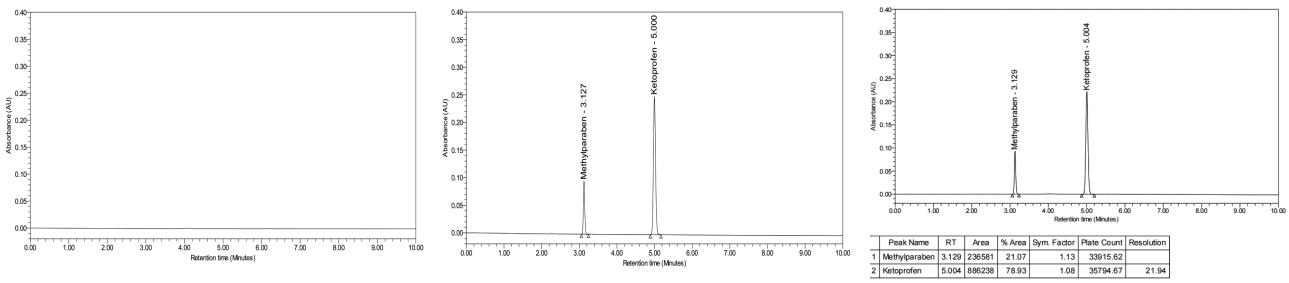
The method was validated for the following parameters: specificity, system suitability, linearity, accuracy, repeatability, and intermediate precision, in accordance with ICH Q2(R2) [9] and AOAC 2016 [10].

3.2.1. Specificity

The diluent, placebo solutions, individual standard solutions of each analyte, mixed standard solution, and test solution were each injected into the chromatographic system. Results are summarized in Table 2.

Table 2. *Results of specificity evaluation*

Chromatogram	t_R methylparaben (min)	t_R ketoprofen (min)	Excipient interference
Mixed standard	3.127	5.000	
Test sample	3.129	5.004	
Placebo 1	Not detected	Not detected	No interference
Placebo 2	Not detected	5.000	No interference
Placebo 3	3.127	Not detected	No interference

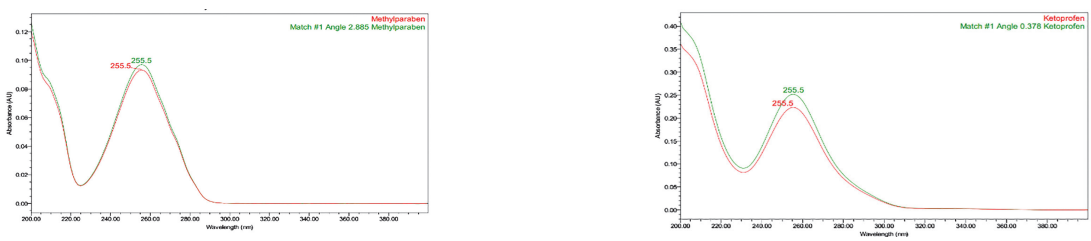


	Peak Name	RT	Area	% Area	Sym. Factor	Plate Count	Resolution
1	Methylparaben	3.129	236581	21.07	1.13	33915.62	
2	Ketoprofen	5.004	886238	78.93	1.08	35794.67	21.94

(a) Placebo 1 *(b) Mixed standard* *(c) Test sample*

Figure 2. *Chromatogram of Placebo 1 (a), Mixed standard (b), and Test sample (c)*

The UV spectra of methylparaben and ketoprofen peaks in the test sample chromatogram were concordant with those in the reference standard chromatogram.



(A) Methylparaben *(B) Ketoprofen*

Figure 3. *Overlay UV spectra of methylparaben and ketoprofen in test sample and reference standard chromatograms*

The results confirmed that the method possesses adequate specificity for simultaneous identification and quantification of methylparaben and ketoprofen in the formulation.

3.2.2. System Suitability

The mixed standard solution (ketoprofen 25 µg/mL; methylparaben 4 µg/mL) was injected six consecutive times. Results are presented in Table 3.

Table 3. *Results of system suitability evaluation*

No.	Methylparaben peak			Ketoprofen peak			Resolution
	t_R (min)	Area (µAU·s)	Symmetry factor	t_R (min)	Area (µAU·s)	Symmetry factor	
1	3.126	244,960	1.08	4.996	1,044,648	1.13	21.52
2	3.126	245,354	1.08	4.998	1,042,632	1.13	21.52
3	3.125	245,391	1.08	4.995	1,040,361	1.13	21.57
4	3.126	244,873	1.08	4.997	1,037,230	1.13	21.58
5	3.127	245,141	1.08	4.998	1,036,918	1.13	21.55
6	3.130	245,705	1.08	5.003	1,037,949	1.14	21.49
Mean	**3.127**	**245,237**	**1.08**	**4.998**	**1,039,956**	**1.13**	**21.54**
RSD (%)	0.06	0.13	–	0.06	0.30	–	–

The RSD of retention times was 0.06% (≤ 1.0%) and the RSD of peak areas for both analytes ranged from 0.13% to 0.30% (≤ 2.0%), all meeting acceptance criteria. The symmetry factors for ketoprofen (1.13) and methylparaben (1.08) were within the acceptable range (≤ 1.5). The F-ratio between consecutive calibration standards was within the range 0.98 – 1.02. The chromatographic system demonstrated adequate suitability for simultaneous analysis of both analytes.

3.2.3. Linearity

Five mixed standard solutions were prepared at concentration levels of 50%, 80%, 100%, 120%, and 150% of the working concentration (ketoprofen: 12.78 – 38.34 µg/mL; methylparaben: 2.025 – 6.075 µg/mL). Results are presented in Table 4.

Table 4. *Results of linearity evaluation*

Parameter	Ketoprofen	Methylparaben
Linear range	12.78 – 38.34 µg/mL	2.025 – 6.075 µg/mL
Regression equation	y = 38564x + 15839	y = 59256x + 4468.1
Correlation coefficient R	0.999 (> 0.998)	0.998 (> 0.998)
Y-intercept (% Y-intercept)	1.6 (≤ 2.0%)	1.8 (≤ 2.0%)

A strong linear correlation was demonstrated between peak area and analyte concentration across the evaluated ranges, with correlation coefficients meeting acceptance criteria (R ≥ 0.998) and percentage y-intercepts ≤ 2.0% for both analytes.

3.2.4. Accuracy

Spiked samples were prepared by adding known amounts of stock standard solutions to 10 mL volumetric flasks containing 1.0 mL of the corresponding placebo solution. Three independent samples were prepared at each concentration level, with a single injection per sample. Results are presented in Table 5.

Table 5. *Results of accuracy evaluation*

Analyte	Concentration level (%)	Amount added (µg/mL)	Mean recovery (%)	RSD (%)
Ketoprofen	80% (n = 3)	19.98 – 20.44	101.6	1.3
	100% (n = 3)	24.86 – 25.63	101.1	0.7
	120% (n = 3)	29.86 – 30.95	101.9	0.8
Acceptance criteria	**98.0 – 102.0%**		**≤ 2.0%**	
Methylparaben	50% (n = 3)	2.00 – 2.05	100. 5	1.6
	100% (n = 3)	50.10 – 50.69	99.0	0.3
	150% (n = 3)	50.10 – 50.69	99.1	0.9
Acceptance criteria	**98.0 – 102.0%**		**≤ 2.0%**	

The method met accuracy requirements: mean percentage recovery for ketoprofen ranged from 101.1 to 101.9%, and for methylparaben from 99.0 to 100.5%; RSD at each concentration level was ≤ 2.0%.

3.2.5. Reporting Range

Based on the linearity and accuracy results, the reporting range of the method was established as 12.78 – 38.34 µg/mL for ketoprofen and 2.025 – 6.075 µg/mL for methylparaben.

3.2.6. Repeatability and Intermediate Precision

Repeatability was assessed using six independently prepared test solutions on the same day. Intermediate precision was evaluated by two analysts on two separate days, with each analyst preparing six samples. Results are presented in Table 6.

Table 6. *Results of repeatability and intermediate precision*

Analyte	Repeatability (n = 6)		Intermediate precision (n = 12)	
	Assay (% label claim)	RSD	Assay (% label claim)	RSD
Ketoprofen	102.6	1.6% (< 2%)	101.8	1.5% (< 3%)
Methylparaben	102.6	1.9% (< 2%)	102.6	1.6% (< 3%)

The method demonstrated satisfactory repeatability and intermediate precision for both analytes: RSD values in all cases met acceptance criteria (≤ 2.0% for each individual analyst; ≤ 3.0% overall).

4. DISCUSSION

To the best of the authors' knowledge, no published method currently exists for the simultaneous quantification of ketoprofen and methylparaben in topical gel formulations, either in Vietnam or internationally. The Vietnamese Pharmacopoeia 5th Edition does not include a dedicated monograph for this preparation. Several published methods for ketoprofen assay employ complex mobile phase conditions or are applicable only to different sample matrices [7, 8]. Methods for methylparaben quantification in the reference literature are typically performed independently or in cosmetic matrices [10].

In the present study, all chromatographic conditions were systematically optimized, including diluent composition, mobile phase ratio, column temperature, and flow rate. The simple isocratic mobile phase system ACN : PA (50 : 50) enabled complete baseline separation of methylparaben ($t_R \approx 3.13$ min) and ketoprofen ($t_R \approx 5.00$ min) within a single analytical run, with a total analysis time of approximately 6 – 7 minutes per injection. The sample preparation procedure is straightforward (sonication, dilution, filtration), requires no liquid-liquid extraction or specialized techniques, and is readily applicable in routine quality control laboratories equipped with standard HPLC instrumentation.

All validation parameters met the acceptance criteria specified by ICH Q2(R2) and AOAC 2016: high linearity ($R \geq 0.998$); mean percentage recovery within 98.0 – 102.0% with RSD ≤ 2.0%; satisfactory repeatability and intermediate precision with RSD ≤ 2.0%. The proposed method is suitable for the simultaneous quality control of the active pharmaceutical ingredient and preservative in ketoprofen topical gel products currently on the Vietnamese pharmaceutical market.

5. CONCLUSION

An HPLC method for the simultaneous quantification of ketoprofen and methylparaben in topical gel has been successfully developed and validated under the following chromatographic conditions: C18 column (4.6 mm × 250 mm, 5 µm); mobile phase: ACN – 0.1% orthophosphoric acid (50 : 50, v/v); flow rate: 1.0 mL/min; column temperature: 35°C; detection wavelength: 254 nm; injection volume: 10 µL. The method has been validated and meets all acceptance criteria in accordance with ICH Q2(R2) and AOAC 2016, demonstrating high precision, accuracy, and a broad linear range. The method is suitable for routine quality control of ketoprofen topical gel formulations currently available on the market.

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