# A Simplified and Rapid High-Performance Liquid Chromatographic Assay for Ketoprofen in Isopropyl Myristate

**Stefan Proniuk, Supaporn Lerkpulsawad,** and **James Blanchard***
Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721

## Abstract

**A high-performance liquid chromatographic (HPLC) procedure for quantitating ketoprofen in isopropyl myristate (IPM), a compound widely used as a receptor medium in drug diffusion studies of topical aqueous-based formulations, is developed. Previously reported HPLC assays for ketoprofen in IPM have employed relatively complex and tedious methods for purifying the IPM prior to injection onto the HPLC column. The present assay method utilizes a direct injection of the IPM-based sample onto a new reversed-phase ODS column and employs ultraviolet detection at 265 nm. Propyl paraben is employed as the internal standard. The mobile phase consists of acetonitrile–methanol–water (36:54:10, v/v/v) at a flow rate of 1.2 mL/min. The calibration curves are linear (correlation coefficient $r \geq 0.988$) over concentration ranges of 0.625–10 µg/mL and 6.25–100 µg/mL. The within-day and between-day precision exhibit coefficients of variation of 1.3–3.3%, and the accuracy (reported as relative error of the mean) varies from –1.9% to 0.6%. The retention times for ketoprofen and propyl paraben are approximately 2.3 and 3.3 min, respectively. The total run time per sample is approximately 7 min. The minimum quantitatable concentration is approximately 0.625 µg/mL. The assay is stability-indicating, rapid, reproducible, sensitive, and readily adaptable for assaying other non-steroidal anti-inflammatory drugs.**

## Introduction

2-(3-Benzoylphenyl) propionic acid (ketoprofen) is one of the most widely prescribed non-steroidal anti-inflammatory drugs (NSAIDs) for the clinical treatment of inflammation and pain relief in rheumatoid arthritis, osteoarthritis, and soft tissue injury (1). There are several different NSAID-containing products available, most being administered orally. However, the extensive first-pass liver metabolism and a relatively high incidence of gastrointestinal side effects associated with ketoprofen and other NSAIDs limits their utility when administered into the systemic circulation (2). Consequently, several alternate routes of administration have been investigated in an attempt to circumvent these problems.

Topical administration is one such route that appears to be promising. Patel and Leswell (3) described several placebo-controlled studies involving topical ketoprofen that indicated that it is an efficacious route that is well tolerated during treatment. The production of topical formulations requires in vitro testing during the developmental stages and monitoring batch-to-batch uniformity of the finished products. Various in vitro techniques have been used to quantitate the amount of drug released from topical vehicles. Two important factors that can regulate the ability of a topical formulation to exert its effects are the diffusibility of drug from the vehicle and its ablility to penetrate the skin barrier (4).

Isopropyl myristate (IPM) has been widely employed as a receptor medium for in vitro release studies (4–9) because it is immiscible with most topical vehicles. In addition, release studies can be performed without using a membrane, and IPM is reported to have properties very similar to those of human skin because of its bipolar nature (9). However, in our own experience and that of others (9), this compound was found to produce changes in the performance characteristics of some of the reversed-phase high-performance liquid chromatography (HPLC) columns commonly used to assay various topical drugs. The column used in this study is a Luna (Phenomenex, Torrance, CA) $C_{18}$ silica-based column which is reported (10) to have a very smooth surface that provides for enhanced stability and longer performance. In addition, the column utilizes a new bonding technique which reportedly provides a more reproducible bonding, virtually no silanol activity, and pH stability over the range of pH 1.5 to 10.

The following is a report on a stability-indicating method for

* Author to whom correspondence should be addressed: James Blanchard, Ph.D., Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721, e-mail: Blanchard@pharmacy.arizona.edu.

quantitating ketoprofen in drug release studies from topical formulations employing a direct injection of the IPM receptor medium. The method is rapid, reproducible, sensitive, and readily adaptable for assaying other NSAIDs. This method does not require preliminary extraction (11) or cleanup of the IPM to remove impurities (9,12) or to avoid the variable results that can occur when using ultraviolet (UV) spectrophotometric assays (6). In addition, it eliminates the need to employ expensive and potentially hazardous radiolabel-based assays (4,5).

## Experimental

### Materials and apparatus

*Instrumentation*

The HPLC system consisted of a Thermo Separation Products (Fremont, CA) model 8815 Isochrom pump, model 9125 injector valve with 20-µL loop (Rheodyne, Cotati, CA) and a Spectra-Physics (San Jose, CA) model 100 variable-wavelength UV detector set at 265 nm. The analytical column was a Phenomenex Luna $C_{18}$ 5-µm column (250 × 4.6 mm) with a Luna 5-µm guard column (30 × 4.6 mm). Peak recordings and integration were accomplished with a Thermo Separation Products model 4290 integrator. All injections were performed at room temperature using a 100-µL Hamilton (Reno, NV) model 710 syringe.

*Drug standard*

Ketoprofen was obtained from Sigma Chemical (St. Louis, MO). All standard samples were dissolved in 99%-pure IPM from Unichema North America (Chicago, IL) to obtain a stock solution of 200 µg/mL.

*Internal standard*

Propyl paraben was obtained from Spectrum Quality Products (Gardena, CA) and prepared as a 200-µg/mL stock solution in IPM.

*Mobile phase*

Acetonitrile was obtained from Burdick and Jackson (Muskegon, MI). HPLC-solvent-grade methanol was from J.T. Baker (Phillipsburg, NJ). The mobile phase was prepared by mixing acetonitrile–methanol–water (36:54:10, v/v/v) and then filtering it through a nylon-66 membrane filter (0.45-µm, Rainin, Woburn, MA). The mobile phase was degassed by sonication before use each day. A mobile phase flow rate of 1.2 mL/min was used.

### Procedures

*Sample preparation*

A stock solution of ketoprofen in IPM was diluted to obtain sample solutions in two concentration ranges. Range I consisted of 0.625, 1.25, 2.5, 5, and 10 µg/mL and Range II consisted of 6.25, 12.5, 25, 50, and 100 µg/mL ketoprofen after combining equal volumes of ketoprofen solution and the internal standard solution. The concentrations of the internal standard solution were 3.85 µg/mL for Range I and 33.33 µg/mL for Range II. This approach ensured that the drug and internal standard peaks were similar in size and helped ensure that maximum sensitivity and reproducibility of the assay was achieved.

*Standard curves*

Five standard curves were prepared for each concentration range. The peak-area ratio of ketoprofen to internal standard (propyl paraben) was plotted against ketoprofen concentration to construct the standard curves. All samples were assayed in duplicate. Duplicate peak areas which differed by less than ± 5% were used in all calculations.

*Between-day and within-day accuracy and precision*

Samples containing 6.25, 25, and 100 µg/mL ketoprofen in IPM were freshly prepared. Eight samples of each concentration were used to assess within-day accuracy and precision over a single day. Between-day accuracy and precision were evaluated by preparing two standard curves per day over 5 consecutive days. The accuracy was assessed by calculating the relative error (RE) of the means for each concentration relative to the known concentration. The coefficient of variation (CV) served as a measure of precision.

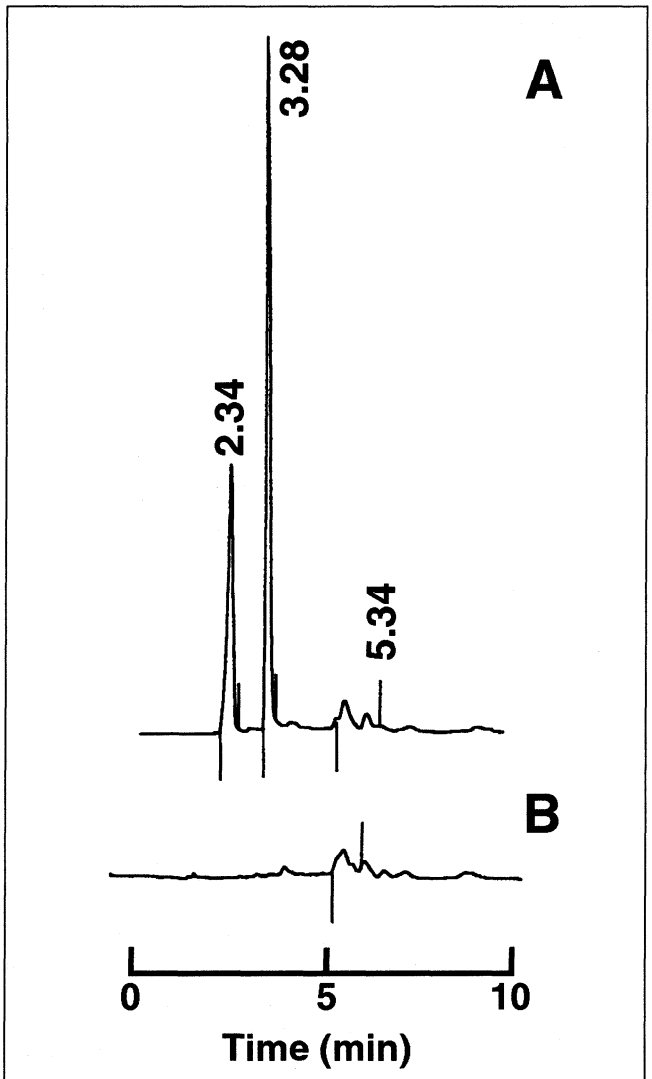


**Figure 1.** Chromatograms of ketoprofen. Ketoprofen (25 µg/mL) and propyl paraben (33.3 µg/mL) in IPM (A) and control (IPM alone) (B).

**Table I. Summary of Calibration Curves for Analysis of Ketoprofen: Range I***

| Standard curve | Slope† | 95%-Confidence limits | $r$ ‡ | Standard error of estimate |
|---|---|---|---|---|
| I | 0.0202 | ± 0.0027 | 0.9995 | 0.0025 |
| II | 0.0204 | ± 0.0047 | 0.9986 | 0.0042 |
| III | 0.0200 | ± 0.0038 | 0.9990 | 0.0035 |
| IV | 0.0212 | ± 0.0128 | 0.9881 | 0.0116 |
| V | 0.0205 | ± 0.0072 | 0.9960 | 0.0065 |
| Mean | 0.0205 | | | |
| SD | 0.0005 | | | |
| CV (%) | 2.23 | | | |

* Range I consisted of concentrations from 0.625 to 10 µg/mL.
† Calculated as $y = mx$.
‡ Correlation coefficient.

**Table II. Summary of Calibration Curves for Analysis of Ketoprofen: Range II***

| Standard curve | Slope† | 95%-Confidence limits | $r$ ‡ | Standard error of estimate |
|---|---|---|---|---|
| I | 0.0225 | ± 0.0167 | 0.9998 | 0.0169 |
| II | 0.0225 | ± 0.0224 | 0.9997 | 0.0203 |
| III | 0.0216 | ± 0.0571 | 0.9979 | 0.0518 |
| IV | 0.0225 | ± 0.0103 | 0.9999 | 0.0093 |
| V | 0.0214 | ± 0.0325 | 0.9993 | 0.0295 |
| Mean | 0.0221 | | | |
| SD | 0.0006 | | | |
| CV (%) | 2.50 | | | |

* Range II consisted of concentrations from 6.25 to 100 µg/mL.
† Calculated as $y = mx$.
‡ Correlation coefficient.

**Table III. Within-Day Accuracy and Precision of Ketoprofen HPLC-Assay**

| Concentration added (µg/mL) | Concentration measured* (µg/mL) | CV (%) | RE† (%) |
|---|---|---|---|
| 6.25 | 6.37 ± 0.19 | 2.91 | –1.92 |
| 25 | 24.98 ± 0.33 | 1.33 | 0.08 |
| 100 | 99.93 ± 2.40 | 2.40 | 0.07 |

* Mean ± SD; $n = 8$.
† Relative error (%) = (true concentration – mean measured concentration) • 100/true concentration.

**Table IV. Between-Day Accuracy and Precision of Ketoprofen HPLC-Assay**

| Concentration added (µg/mL) | Concentration measured* (µg/mL) | CV (%) | RE† (%) |
|---|---|---|---|
| 6.25 | 6.34 ± 0.15 | 2.36 | –1.44 |
| 25 | 24.91 ± 0.33 | 1.32 | 0.36 |
| 100 | 99.40 ± 3.30 | 3.32 | 0.60 |

* Mean ± SD; $n = 10$.
† Relative error (%) = (true concentration – mean measured concentration) • 100/true concentration.

*Column care*

For overnight storage, the mobile phase flow rate was reduced to 0.1 mL/min. For more prolonged storage, the column was washed with methanol for approximately 1 h at a flow rate of 1 mL/min. The flow of mobile phase was then stopped, and the column was stored in methanol.

## Results and Discussion

Calibration curves prepared in two ketoprofen concentration ranges (0.625–10 µg/mL and 6.25–100 µg/mL) demonstrated the satisfactory and consistent behavior of the method. Figure 1 shows the chromatograms for ketoprofen and the internal standard in IPM and for blank IPM. It is evident that impurities contained in IPM (mostly long-chain fatty acids) do not cause any interference with the ketoprofen peaks, and both ketoprofen and the internal standard are well resolved. The run time per assay averaged about 7 min. The data from the low- and high-concentration ranges were analyzed using least-squares regression analysis, and the results are shown in Tables I and II, respectively. All of the calibration curves were linear with a correlation coefficient $r \geq 0.988$. The intercepts were not significantly different from zero; therefore, the least-squares regression line was forced through the origin. In addition, it can be observed that the calibration curves for the low- and high-ketoprofen concentration ranges were in good agreement. The standard error of the estimate for each calibration curve is also listed because it provides a measure of the difference between the experimental and calculated values and thus a measure of the scatter of the data about the best-fit line.

The stability-indicating nature of the assay was verified by collecting several fractions of the eluate from the chromatograph corresponding to the elution time of the ketoprofen peak. The eluates were then combined and analyzed using electrospray mass spectrometry (Finnigan MAT model TSQ 7000, San Jose, CA) in the negative ion mode. The analysis confirmed the identity and uniqueness of the ketoprofen peak.

The within-day and between-day assay accuracy and precision as measured by the RE and CV are summarized in Tables III and IV, respectively. The RE varied from 0.07% to –1.92% for the within-day assays and from 0.60% to –1.44% for the between-day assays. The CV varied from 1.33% to 2.91% for the within-day assays and from 1.32% to 3.32% for the between-day assays. These results indicate that the assay was accurate and reproducible.

The limit of quantitation is defined as the amount of drug that can be quantitated with acceptable precision and accuracy under the experimental conditions with a signal-to-noise ratio of at least 10 (13). The lower limit of quantitation of this assay is approximately 0.625 µg/mL.

## Conclusion

The method reported here is the first analytical HPLC procedure that is suitable for quantitating ketoprofen in the presence

of IPM and requires no cleanup of the sample prior to injection onto the chromatograph. In addition, the method utilizes a direct injection of the sample and employs isocratic elution at ambient temperature. As the data illustrate, this assay is rapid, precise, accurate, and simple to perform. Shah et al. (14) noted that in order to maintain sink conditions in the receptor medium when assessing drug release from topical formulations, the drug should be sufficiently soluble so that it can be quantitated in a relatively small sample size. The high sensitivity of this assay requires only a very small (20 µL) injection volume for the accurate and precise analysis of ketoprofen. In addition, the calibration curves for the low- and high-concentration ranges are in good agreement, thus sample concentrations can be determined accurately and precisely over a relatively broad concentration range using a small (20 µL) sample size. The slightly lower (approximately 7%) slope in the low-concentration range indicates a small degree of non-linearity in the assay, the cause of which is unknown. The small RE and the reproducibility of both the within-day and between-day assays indicate that a single calibration curve can be used reliably over a time period of at least 5 working days without the need to run daily standards concurrently. With some columns and mobile phases, it was observed that the ketoprofen peaks were not reproducible after only two injections. Using the protocols described here, there was no evidence of deterioration of the assay using the same column over a 3-month period. Measurement of the diffusion of ketoprofen from a gel formulation into an IPM receptor medium determined by this method is greatly simplified in comparison to previous assays. In our experience, this assay proved to be suitable for monitoring the release of ketoprofen from several poloxamer 407 gel formulations and should greatly facilitate the evaluation of the release pattern of ketoprofen from similar topical formulations.

## Acknowledgments

The authors are grateful to Unichema North America for supplying the samples of isopropyl myristate.

## References

1. O. Airaksinen. Ketoprofen 2.5% gel versus placebo gel in the treatment of acute soft tissue injuries. *Int. J. Clin. Pharm.* **31:** 561–63 (1993).
2. R.O. Day, G.G. Graham, D.E. Furst, and E. Lee. *Drugs for Rheumatic Disease: Ibuprofen, Fenoprofen, Ketoprofen,* H.E. Paulus, Ed. Churchill Livingstone Inc., New York, NY, 1987, pp 315–45.
3. R.K. Patel and P.F. Leswell. Comparison of ketoprofen, piroxicam, and diclofenac gels in the treatment of acute soft-tissue injury in general practice. *Clin. Ther.* **18:** 497–507 (1996).
4. J. Ostrenga, C. Steinmetz, and B. Poulsen. Significance of vehicle composition I: relationship between topical vehicle composition, skin penetrability, and clinical efficacy. *J. Pharm. Sci.* **60:** 1175–79 (1971).
5. B.J. Poulsen, E.Young, V. Coquilla, and M. Katz. Effect of topical vehicle composition on the in vitro release of fluocinolone acetonide and its acetate ester. *J. Pharm. Sci.* **57:** 928–33 (1968).
6. P. Chen-Chow and S.G. Frank. The determination of lidocaine and benzocaine in isopropyl myristate. *Int. J. Pharm.* **8:** 81–87 (1981).
7. S.C. Chi and H.W. Jun. Release rates of ketoprofen from poloxamer gels in a membraneless diffusion cell. *J. Pharm. Sci.* **80:** 280–83 (1991).
8. F.P. Bonina, L. Montenegro, and F. Guerrera. Naproxen 1-alkylazacycloalkan-2-one esters as dermal prodrugs: in vitro evaluation. *Int. J. Pharm.* **100:** 99–105 (1993).
9. E.W. Smith and J.M. Haigh. In vitro diffusion cell design and validation. I. A stability-indicating high-performance liquid chromatographic assay for betamethasone 17-valerate in purified isopropyl myristate receptor phase. *Pharm. Res.* **6:** 431–35 (1989).
10. Phenomenex Chromatography 98/99 Catalog. Phenomenex, Torrance, CA, pp 146–47.
11. S.-C. Chi and H.W. Jun. Quantitation of ketoprofen in isopropyl myristate by high performance liquid chromatography. *J. Liq. Chromatogr.* **12:** 2931–45 (1989).
12. Personal communication, Hanson Research Corporation, Chatsworth, CA, 1997.
13. W.J. Lough and I.W. Wainer. *High Performance Liquid Chromatography: Method Development and Quantitation.* Blackie Academic & Professional, Glasgow, U.K., 1996, pp 160–61.
14. V.P. Shah, J.S. Elkins, and R.L. Williams. In vitro drug release measurement for topical glucocorticoid creams. *Pharmacopeial Forum* **19:** 5048–60 (1993).

Manuscript accepted August 10, 1998.