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Development and validation of a stability-indicating high-performance liquid chromatographic assay for ketoprofen topical penetrating gel

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Abstract

An isocratic RP-HPLC procedure has been developed and validated for the quantitative determination of ketoprofen in a topical gel. The HPLC procedure consist of a YMC ODS-AQ, 5-μm particle size analytical column (150 mm × 4.6 mm); Alltech Econosphere C_{18}, 5-μm particle size guard column; detection at 233 nm; 1 ml/min flow rate; 20-μl injection volume. The mobile phase consisted of pH 3.5 phosphate buffer–water–acetonitrile (8:43:49, v/v). Sample preparation was a simple extraction of ketoprofen with mobile phase. The above conditions resolved and eluted ketoprofen, excipients, and potential degradants within 35 min, with ketoprofen eluting at about 6.5 min. The procedure was validated with respect to specificity, accuracy, precision, and linearity. The accuracy of the procedure, determined by spike recovery measurements, was 100.1–100.5%. The intra- and inter-day precisions were demonstrated by the relative standard deviations (RSD) of 0.3–0.6% and 0.5%, respectively. The intermediate precision was determined by comparing the results obtained with four independently prepared samples by two chemists using two columns on different days. The results indicate no significant difference ($P = 0.17$). The procedure showed linearity over the concentration range 4×10^{-5} to 1×10^{-1} mg/ml.

Keywords: Ketoprofen; Topical gel; Stability studies

1. Introduction

Ketoprofen (Fig. 1) is a non-steroidal anti-inflammatory drug (NSAID), a group of non-narcotic drugs used to relieve mild to moderate pain. The drug is effective in relieving pain associated with rheumatic and non-rheumatic inflammatory disorders, vascular headaches, and dysmenorrhea. Ketoprofen comes as oral capsules or tablets, rectal suppositories, and as extended-release tablets or capsules [1]. Serious side effects that involve the gastrointestinal system can occur with ketoprofen and other NSAID therapy. The drug can cause ulcerations, abdominal burning, pain, diarrhea, gastritis, and even serious gastrointestinal bleeding when taken orally [1,2].

Several topical preparations have been shown to be effective and at the same time avoid the adverse effects of oral delivery of the drug. Ketoprofen cream administered to the gingival for treatment of periodontal disease [3], percutaneous absorption of ketoprofen from creams [4], and the effect of ketoprofen cream on osteopenia [5] have been studied. Also, the effect of different ointment bases on the efficacy of ketoprofen topical preparations [6,7] and transdermal delivery of the drug from a new soya-lecithin aggregate, have been studied [8].

Clinical effectiveness of topical ketoprofen preparations have also been demonstrated. An intra-oral gel have been shown to prevent tension-type headache and migraine [9,10], a cream applied on the anterior skin of the neck reduced postoperative sore throat [11], a gel administered into oral surgical sites produced greater analgesic effect [12], and a transdermal ketoprofen delivery system has been shown to be effective in reducing delayed onset muscle soreness [13].

Topical formulations of ketoprofen are however, not commercially available and are usually obtained from compounding pharmacies. Ketoprofen topical penetrating (KTP) gel is an example of such a formulation. The formulation contains 1% (w/w) ketoprofen in a poloxamer (Pluronic F-127) gel. Other excipients in the gel include soya-lecithin, isopropyl palmitate, alcohol, sorbic acid, and a pH 5.0

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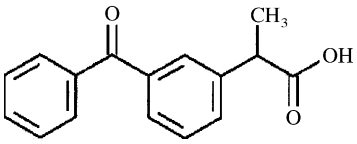

Fig. 1. Chemical structure of ketoprofen ((±)-*m*-benzoylhydratropic acid).

citrate–phosphate buffer. The United States Pharmacopeia is considering introducing a monograph for this compounded formulation. A validated analytical procedure capable of determining ketoprofen in the presence of the above excipients and also in the presence of potential degradation products would be needed for the monograph. A search through the literature, however, revealed few published analytical procedures for determining ketoprofen in topical preparations. Ketoprofen as the drug substance is usually assayed by simple non-specific titrimetric procedures [14,15]. Published analytical procedures for determining ketoprofen in topical preparations include a FT-near infrared (NIR) spectroscopy procedure [16], a solid-phase extraction (SPE)-UV spectrophotometric procedure [17], and an HPLC procedure [18]. The FT-NIR procedure was not demonstrated to be stability indicating. The SPE-UV procedure is reported to give inflated assay results due to non-specific matrix absorption. Derivative spectroscopy was required to resolve this problem. The HPLC procedure was developed for ketoprofen in a gel formulation that contained only one excipient. In addition, the precision for the procedure ranged from 1.3% to 3.3%. This report presents a simple isocratic HPLC procedure, which resolved ketoprofen from the excipients and potential degradants, and afforded an accurate and precise quantitation of ketoprofen in the KTP gel.

2. Experimental

2.1. Materials

Ketoprofen (USP grade), isopropyl palmitate, sorbic acid, soya-lecithin, citric acid, and Pluronic F-127 were purchased from Gallipot (St. Paul, MN, USA). Benzoic acid reference standard and ketoprofen reference standard were obtained from The United States Pharmacopeia (Rockville, MD, USA) and were dried under the conditions specified on the vials. Dibasic sodium phosphate, monobasic sodium phosphate, and 30% hydrogen peroxide solution were purchased from Fisher (Fair Lawn, NJ, USA). Phosphoric acid (85%) and hydrochloric acid were obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA) and acetonitrile was purchased from Burdick & Jackson (Muskegon, MI, USA). All chemicals were reagent grade or better. The 0.45-μm Millex-HV filter was purchased from Millipore (Billerica, MA, USA). The KTP gel and the placebo gel were provided by Kaye's Pharmacy, Baltimore, MD, USA. Low-actinic glassware was used for all solutions containing ketoprofen.

2.2. Instrumentation

Two HPLC systems were used. The first one, a Hewlett Packard 1100 Series HPLC system consisted of an HP G1322A degasser, an HP G1311A quaternary HPLC pump, an HP G1313A autosampler, an HP G1316A column oven, an HP G1314A UV detector, and ChemStation software version A.04.01. A reversed phase analytical column, 150 mm × 4.6 mm packed with 5 μm, YMC ODS-AQ (Waters, Milford, MA, USA) and a guard column, 4.6 mm × 5 mm packed with 5 μm, Econosphere C_{18} (Alltech, Deerfield, IL, USA) were used on this system. A second HPLC system, that was used for determining the intermediate precision, consisted of a Thermo Separations Products (Piscataway, NJ) P4000 pump, SCM100 vacuum degasser, AS 3000 autosampler, model UV6000LP diode array detector, and ChromQuest software, version 2.51. A 150 mm × 4.6 mm column containing 5 μm Alltech Nucleosil 100 C_{18} packing, and a 5 mm × 4.6 mm column containing 5 μm Econosphere C_{18} packing (Alltech), were the analytical and guard columns, respectively.

Other equipment used included a Chromato-Vue C-70G UV viewing cabinet from UVP (Upland, CA, USA); an ABU93 Triburette station and a VIT90 Video Titrator from Radiometer Analytical (Villeurbanne, France); an RTE –9DD Refrigerated Circulating Bath from Neslab (Portsmouth, NH, USA); and a Reacti-Therm Heating/Stirring module from Pierce (Rockford, IL, USA).

2.3. Chromatographic conditions

The isocratic HPLC separation was carried out with pH 3.5 phosphate buffer–water–acetonitrile (8:43:49, v/v) at a flow rate of 1.0 ml/min. The pH 3.5 phosphate buffer was prepared by dissolving 3.4 g of monobasic potassium phosphate in water, adjusting the pH to 3.5 with phosphoric acid, and diluting with water to 1 l. Column temperature was ambient and an injection volume of 20 μl was used. Data were collected from 200 to 360 nm and peak–area responses of chromatograms extracted at 233 nm were used for all quantitative work.

2.4. Standard preparation

About 34 mg of ketoprofen reference standard were accurately weighed and dissolved in the mobile phase to make 100 ml solution. A 15-ml aliquot of the resulting solution was further diluted to 100 ml with mobile phase.

2.5. Test preparation

About 500-mg, accurately weighed, portion of the KTP gel or placebo gel (blank) was dispersed by sonication for 10 min in 50 ml of mobile phase and diluted to 100 ml with the mobile phase. The solution was filtered using a 0.45-μm Millex-HV filter membrane discarding the first 15 ml.

2.6. Filter check

Test preparation and blank prepared as in Section 2.5 were compared with samples prepared by centrifugation at 1500 rpm for 10 min at room temperature.

2.7. Stress conditions

The validation (specificity) was carried out using stress-degraded samples. Samples of the KTP gel and placebo gel were stressed under the following conditions, and test preparations were prepared using the stressed samples.

2.7.1. Light exposure

KTP gel samples were spread as thin layers on the inside walls of two quartz UV cells. The cells were exposed uncovered, sample side up, to UV (254 and 365 nm) and white light in a UVP Chromato-Vue C-70G light chamber for 1 or 2 h.

2.7.2. Heat exposure

The KTP gel samples were transferred into 10-ml Reacti-vials, covered, and heated at 90 °C for 4 h in a Reacti-Therm Heating/Stirring module with occasional stirring.

2.7.3. Oxidative stress

A 30% hydrogen peroxide solution was mixed with a portion of the KTP gel in amber plastic bottles to give a peroxide concentration of about 3%. The peroxide-stressed gel was sampled after 4 and 26 h of storage.

2.7.4. Acid exposure

A portion of the KTP gel was adjusted to about pH 2 with dilute hydrochloric acid and stored in the dark. Samples were taken for analysis after 4 and 26 h.

2.7.5. Base exposure

A portion of the KTP gel was adjusted to about pH 9 using 0.5% sodium hydroxide solution, stored in the dark, and sampled for analysis after 4 and 26 h.

2.8. Peak purity

The photodiode array detection and the chromatographic software were used to assess the purity of the ketoprofen peak. The software use similarity curves which compare the selected spectrum against all the spectra across the peak and threshold calculated based on the signal-to-noise ratio, to determine peak purity. If the purity ratio, which is calculated using the similarity and threshold data is less than 1, the peak is considered to be spectrally pure.

2.9. Titrimetric assay of ketoprofen drug substance

The ketoprofen drug substance that was used to prepare the KTP gel was assayed using the USP ketoprofen assay procedure [14]. An accurately weighed portion (about 200 mg) of the ketoprofen drug substance was dissolved in 25 ml of ethyl alcohol, and diluted with 25 ml of water. The solution was titrated with 0.1 M sodium hydroxide solution that has been standardized with benzoic acid. The endpoint was determined potentiometrically. A blank determination was performed and used to correct for the volume of 0.1 M sodium hydroxide.

3. Results and discussion

3.1. Assay of ketoprofen drug substance

The ketoprofen drug substance was assayed using the USP ketoprofen assay procedure [14]. In three titrations, the average percentage of ketoprofen in the drug substance was 101.0 (relative standard deviation (RSD) 0.8%).

3.2. Validation

The procedure is intended for an assay which is described as a Category I procedure in General Chapter ⟨1225⟩ *Validation of Compendial Methods*, of the US Pharmacopeia [14], and hence the data elements required for validation are specificity, accuracy, precision, and linearity.

3.2.1. Specificity

3.2.1.1. Sample clean up and filter check. The ketoprofen in a 500-mg sample of the KTP gel was extracted into 100 ml of the mobile phase by sonication and vigorous shaking. A filtered portion of the extract was injected and the results were compared with a centrifuged sample. Chromatograms extracted at 233 nm showed no significant difference between the peak–area responses of ketoprofen in the filtered or

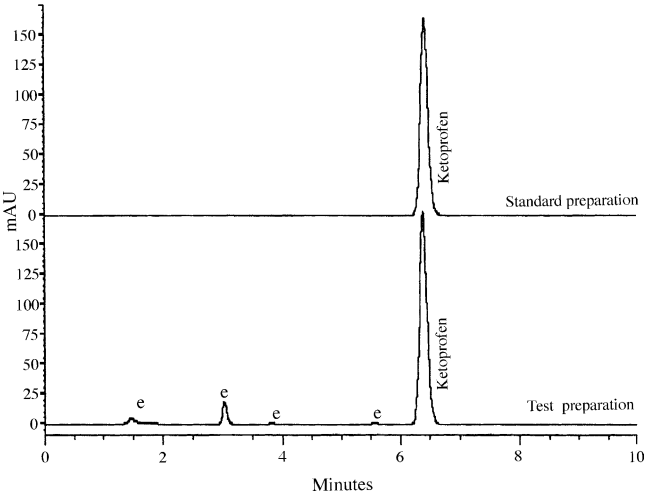

Fig. 2. Chromatograms of the standard preparation and test preparation. Chromatographic conditions: column: YMC ODS-AQ, 150 mm × 4.6 mm, 5-μm packing; mobile phase: pH 3.5 phosphate buffer–water–acetonitrile (8:43:49, v/v) at a flow rate of 1.0 ml/min; injection volume: 20 μl; detection: 233 nm. The excipient peaks are denoted by "e" in the chromatogram.

Table 1
Summary of the performance parameters of the HPLC procedure for ketoprofen topical penetrating gel

Parameter	Value	Comments
Efficiency (theoretical plates)	11600	Standard preparation (0.05 mg/ml)
Tailing factor (USP)	1.1 (RSD = 3%, $n = 6$)	Measured at width of peak at 5% of peak height at mg/ml
Linearity		
Range	4×10^{-5}–1×10^{-1} mg/ml	
Correlation coefficient	1.0000	
Equation:		
Intercept	3261 (S.E. = 3471)	Intercept was not significantly different from 0 (95% CL). S.E. is standard error
Slope	2.95×10^{7} (S.E. = 6.5×10^{4})	
Precision		
Injection repeatability	0.1–0.4%	$n = 6$; n is the number of injections or samples
Repeatability (intra-day)	0.3–0.6%	$n = 3$
Repeatability (inter-day)	0.5%	$n = 3$
Recovery		
70% Spiked	100.1% (RSD = 0.3%, $n = 3$)	
100% Spiked	100.4% (RSD = 0.2%, $n = 3$)	
120% Spiked	100.5% (RSD = 0.4%, $n = 3$)	
Solution stability	No significant change in peak response over a 15-h period	

centrifuged samples. Analysis of similarly treated placebo gel showed no peak in the ketoprofen locus in the chromatograms of the filtered and centrifuged samples.

Fig. 2 shows that the retention time (∼6.5 min) of the primary peak in the chromatogram of the KTP gel, ketoprofen, is the same as the retention time of the ketoprofen peak in the standard, indicating specificity of the procedure. Furthermore, samples of the KTP gel that had been stressed under ultraviolet and white light were analyzed and the chromatograms are shown in Fig. 3. The chromatograms extracted at 233 nm exhibited eight new peaks after 2 h exposure to the UV (254 and 365 nm) and white light. None of the eight peaks was observed in chromatograms of the control sample that was prepared with KTP gel but not exposed to the stress condition, indicating that the eight peaks, identified in Fig. 3 are due to degradants. Also, none of the placebo samples, both stressed and non-stressed, showed a peak at or near the retention time of ketoprofen. The results show that

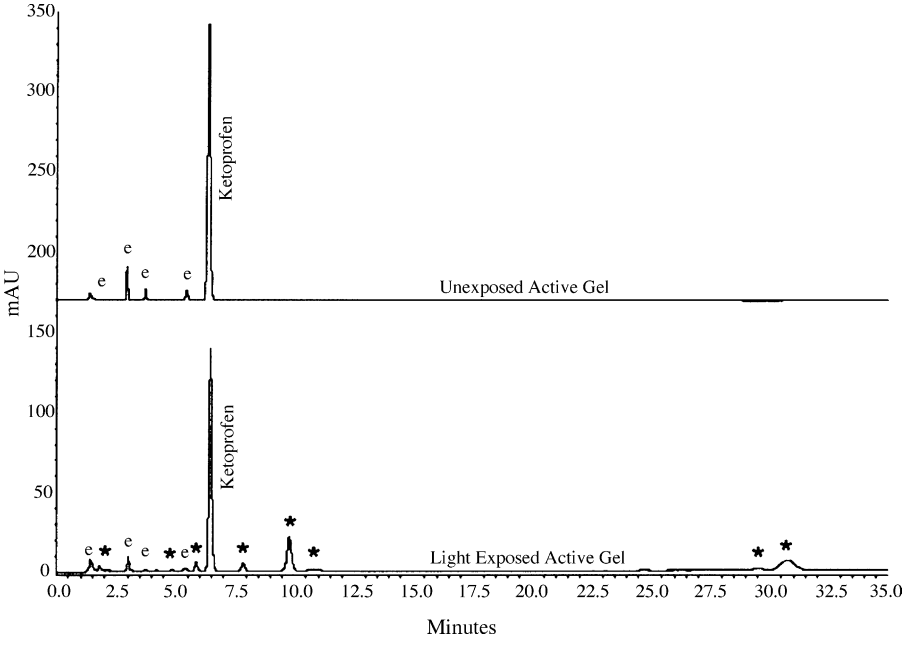

Fig. 3. Chromatogram of *test preparation* made from ketoprofen topical penetrating gel that has been exposed to UV (254 and 365 nm) and visible light for 2 h. Chromatographic conditions as in Fig. 2. The degradants peaks are denoted by "✱" and excipient peaks are denoted by "e" in the chromatogram.

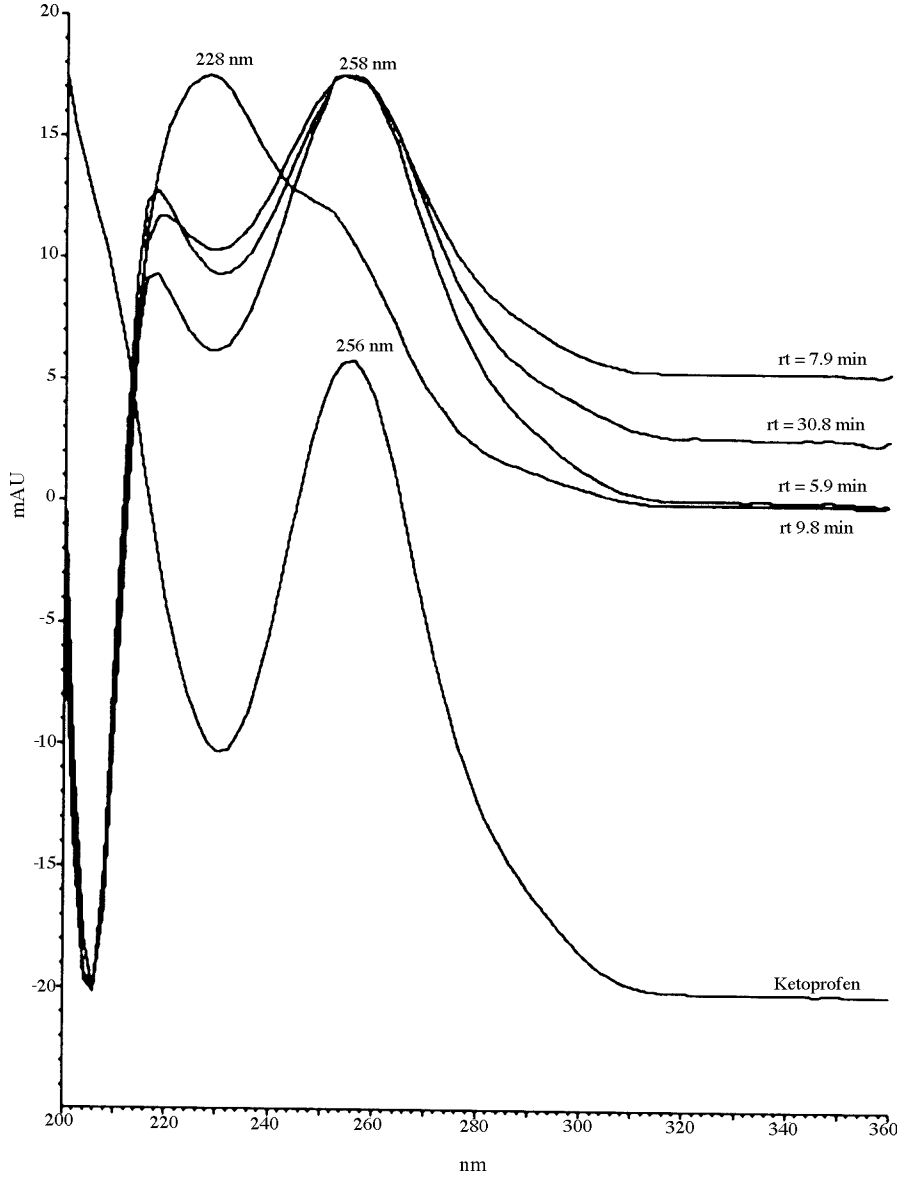

Fig. 4. Spectra of the ketoprofen peak and four major degradation products in the chromatogram of light-exposed ketoprofen topical penetrating gel (Fig. 3). "rt" in the figure denotes retention time.

the HPLC conditions resolved and eluted ketoprofen, excipients, and the potential degradant peaks within 35 min. Also, from injections of the standard preparation, the column efficiency was at least 11,600 theoretical plates and the tailing factor for the ketoprofen peak was 1.1 (RSD 3%), as shown in Table 1.

The UV spectrum of the ketoprofen peak and that of three degradation products showed absorption maxima at about 256 nm (Fig. 4). However, at this wavelength, the minor degradation products were not detected. Hence, the wavelength of 233 nm at which both ketoprofen and the degradation products are detectable, was chosen.

There were no significant changes in the chromatograms obtained with KTP gels subjected to oxidative, heat, acid or base stress, when compared to the chromatograms obtained with the non-stressed samples. The physical appearance of the gel, however, changed when it was subjected to heat stress—the gel appeared to have cracked and a layer of liquid appeared on the top.

The purity of the ketoprofen peak was assessed with the ChemStation software (see Section 2.8). The purity ratio was less than 0.5 in all cases, indicating the peak was spectrally pure.

3.2.2. Accuracy

The accuracy of the procedure was demonstrated by the recovery studies, which were carried out by spiking samples of placebo gel with ketoprofen drug substance solution in alcohol to give concentrations equivalent to 70%, 100%, and 120% of the topical penetrating gel. Three samples were

prepared at each concentration and assayed against standard preparations. As shown in Table 1, recoveries were 100.1–100.5% (RSD 0.2–0.4%). A sample of the placebo gel was also directly spiked with ketoprofen (powder) at 100% of the active concentration. The average recovery was 100.6% (RSD 1.1%). The larger relative standard deviation in this case may be due to homogeneity problems in mixing the dry ketoprofen powder into the gel instead of adding it as a solution.

3.2.3. Precision

3.2.3.1. Injection repeatability. The relative standard deviation of the ketoprofen peak–area in six replicate injections of the standard preparation determined each day for three consecutive days ranged from 0.1% to 0.4%.

3.2.3.2. Assay repeatability. Assay of the KTP gel was performed in triplicate each day for three consecutive days. The precision of the assays on each of the three days ranged from 0.3% to 0.6%, and the overall precision was 0.5%. The mean of the assay results for the three days was 94.1% of the label amount of ketoprofen (Table 2).

3.2.3.3. Intermediate precision. Another chemist, using a different HPLC system, a different brand of C_{18} column (see Section 2.2), on a different day, assayed the KTP gel. The results show that the gel contained 94.2% of the label amount of ketoprofen (RSD 0.01%) (Table 2). The results obtained by the two analysts using two different columns on different days were not significantly different ($P = 0.17$).

3.2.4. Linearity and range

Standard solutions were prepared in concentrations ranging from 4×10^{-5} to 1×10^{-1} mg/ml, which corresponds to 0.1–200% of the *standard preparation* concentration. By peak–area response, the correlation coefficient $r = 1.0000$, for the ketoprofen peak. The intercept was not significantly different from 0 at the 95% confidence level (Table 1).

Table 2
Comparison of the assay data obtained for the ketoprofen topical penetrating gel

	Day 1	Day 2	Day 3	Second LC system/analyst[a]
Assay (percentage ketoprofen content)				
Sample 1	94.2	93.6	93.6	94.2
Sample 2	94.1	93.6	94.5	94.2
Sample 3	94.5	93.3	94.6	94.2
Average	94.4	93.6	94.2	94.2
SD	0.5	0.3	0.6	0.01
RSD (%)	0.5	0.3	0.6	0.01

There was no significant difference ($P = 0.17$) between the data obtained on different days by the same analyst and data generated by a second analyst using a different LC system and a different brand of C_{18} column.

[a] Assay by a different analyst using a different LC system and a different brand of C_{18} column.

3.2.5. Solution stabilities of standard and assay preparations

The solution stabilities of the *standard* and *assay preparations* were investigated by making injections of each solution over a period of 15 h. No significant change in the peak–area responses were observed for the *standard preparation* and the *assay preparation* over the 15-h period.

4. Conclusion

This report presents development and validation of a simple isocratic HPLC procedure suitable for the analysis of ketoprofen in a topical gel. The precision of the current procedure was less than 0.6%, significantly better than that (1.3–3.3%) reported previously for another HPLC procedure [18]. Sample clean up was a simple extraction with mobile phase, and unlike a previously published procedure [17], no matrix related problems were encountered. The current HPLC procedure separated the excipients and potential degradant peaks from the ketoprofen peak and has been demonstrated to be sufficiently accurate, specific, precise, and stability indicating, and is suitable for the analysis of ketoprofen in compounded ketoprofen topical penetrating gel.

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