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Short communication

Simultaneous HPLC determination of ketoprofen and its degradation products in the presence of preservatives in pharmaceuticals

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Abstract

A novel and quick high-performance liquid chromatography (HPLC) method with UV spectrophotometric detection was developed and validated for the determination of five compounds in topical gel. The described method is suitable for simultaneous determination of active component ketoprofen, two preservatives methylparaben and propylparaben and two degradation products of ketoprofen—3-acetylbenzophenone and 2-(3-carboxyphenyl) propionic acid—in a topical cream after long-term stability tests using ethylparaben as an internal standard.

The chromatographic separation was performed on a 5 μm Supelco Discovery C18 column (125 mm × 4 mm i.d., Sigma–Aldrich); the optimal mobile phase for separation of ketoprofen, methylparaben, propylparaben, degradation products 3-acetylbenzophenone and 2-(3-carboxyphenyl) propionic acid and ethylparaben as internal standard consists of a mixture of acetonitril, water and phosphate buffer pH 3.5 (40:58:2, v/v/v). At a flow rate of 1.0 ml min^{-1} and detection at 233 nm, the total time of analysis was less than 10 min.

The method was applied for routine analysis (batch analysis and stability tests) of these compounds in topical pharmaceutical product.

Keywords: HPLC; Ketoprofen; Pharmaceuticals; Degradation products; Methylparaben; Propylparaben

1. Introduction

Ketoprofen (2-(3-benzoylphenyl)propionic acid, KP, Fig. 1) is one of the most widely prescribed non-steroidal anti-inflammatory drugs (NSAIDs). A new pharmaceutical preparation (gel) containing ketoprofen as an active compound with anti-inflammatory and analgesic activity was developed for treatment of diseases of the muscolo-skeletal apparatus, in which a local action is preferred. In order to prevent bacterial growth during the storage of the formulation [1,2], two commonly used preservatives—a mixture of the methylester and propylester of *p*-hydroxybenzoic acid (methylparaben (MP) and propylparaben (PP) (Fig. 1))—have been used.

Recently, there have been a number of reports dealing with various analytical methods for the determination of ketoprofen, such as capillary electrophoresis [3–5], capillary isotachophoresis [6], combined use of headspace solid-phase micro-extraction and isotopic dilution [7], gas chromatography–mass spectrometry (GC–MS) [8], capillary electrochromatography [9,10], high-performance liquid chromatography (HPLC) [1,11–13], HPLC–MS [14,15] or micellar chromatography [16] as well.

Only one HPLC method has been found in literature [17] for simultaneous determination of KP and its degradation products, but not in the presence of preservatives. Recently, preservatives in pharmaceuticals have to be quantified. HPLC analysis of MP and PP is frequently described in the literature [18–20]; another publication deals with simultaneous quantification of ketoprofen and parabens in a commercial gel formulation by RP–HPLC with UV detection [2], but there is

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no any HPLC method describing simultaneous determination of all five components—ketoprofen, two degradation products 3-acetylbenzophenone (ABP) and 2-(3-carboxyphenyl) propionic acid (CPA), MP and PP—in pharmaceutical preparations. The British Pharmacopoeia 1993, Addendum 1995 [21] specify a HPLC method for the determination of KP and its degradation products ABP and CPA, but not simultaneously in the presence of MP or PP. Moreover, the pharmacopoeial method does not use internal standard, which in case of sample preparation from matrixes like gels or ointments would be desirable.

For such a formulation, a novel method capable to analyse simultaneously the active component ketoprofen, its two degradation products—3-acetylbenzophenone and 2-(3-carboxyphenyl)propionic acid (Fig. 1)—and two preservatives methylparaben and propylparaben was developed. Thereafter, this HPLC method was successfully applied for the separation, quantification and stability study of all these compounds in the commercially available pharmaceutical formulation Ketoprofen gel 2.5%.

2. Experimental

2.1. Reagents

The in-house secondary standard of KP used during this study was obtained from MD-Pharm (Kravare, Czech Republic); standards of degradation products ABP and CPA were obtained from European Pharmacopoeia authority (Strasbourg, France), methylparaben and propylparaben from IVAX (Opava, Czech Republic); internal standard ethylparaben was obtained from Sigma–Aldrich Co. (Prague, Czech Republic). All compounds have been assayed against the EP reference standards. Standard solutions were prepared in acetonitrile. The final concentrations of the sample or reference standards were about 625 $\mu g\,ml^{-1}$ of ketoprofen, 25 $\mu g\,ml^{-1}$ of methylparaben, 12.5 $\mu g\,ml^{-1}$ of propylparaben, 5 $\mu g\,ml^{-1}$ of 3-acetylbenzophenone and 2-(3-carboxyphenyl)propionic acid and 10 $\mu g\,ml^{-1}$ of internal standard ethylparaben.

Acetonitrile (Chromasolv, for HPLC) was obtained from Sigma–Aldrich Co. (Prague, Czech Republic), *ortho*-phosphoric acid 85% and natrium dihydrogen phosphate from Merck (Darmstadt, Germany). All other chemicals were reagent grade from Merck (Darmstadt, Germany).

Ketoprofen gel 2.5% was supplied from Herbacos–Bofarma Ltd. (Bochemie Group, Pardubice, Czech Republic); the declared amount of MP is 0.10 g in 100 g and of PP is 0.05 g in 100 g of gel.

The deionised water was purified by a Milli-Q system (Millipore, MA, USA) and meets the European Pharmacopoeia specifications.

2.2. Chromatographic system

The LC system, consisting of a binary pump LCP 4100 (Ecom, Prague, Czech Republic), Waters autosampler 717 plus, variable wavelength UV detector Waters 486 (Waters, Milford, MA) and a PC for data processing, was controlled by

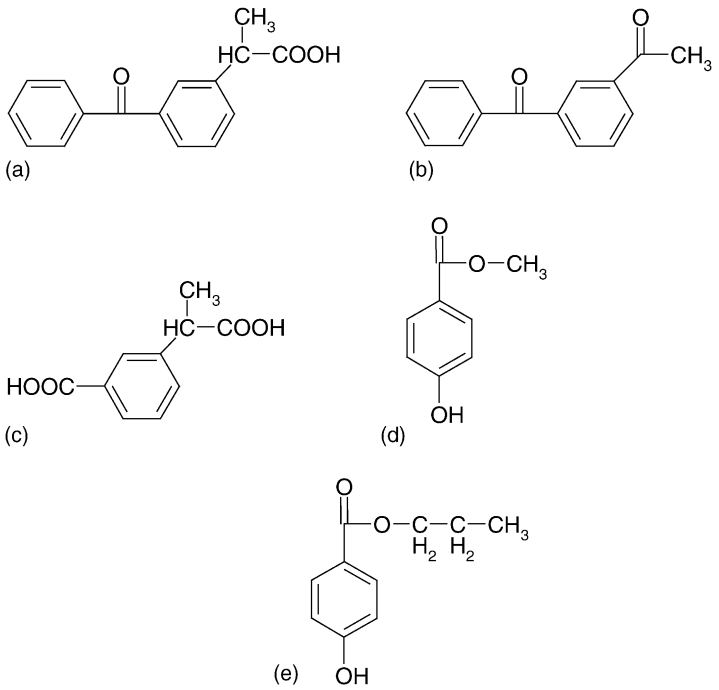

Fig. 1. Major components, degradation products and preservatives in Ketoprofen gel 2.5%: (a) ketoprofen (KP); (b) 3-acetylbenzophenone (ABP); (c) 2-(3-carboxyphenyl)propionic acid (CPA); (d) methylparaben (MP); (e) propylparaben (PP).

Table 1
Peaks resolution

Flow rate (ml min^{-1})	0.6	1.0	1.2	1.5	2.0
CPA–methylparaben	2.816	3.417	3.266	3.009	2.611
Methylparaben–ethylparaben	4.374	4.289	3.827	3.490	3.174
Ethylparaben–propylparaben	6.407	7.323	6.414	5.994	5.407
Propylparaben–ketoprofen	2.734	3.160	2.381	2.293	2.134
Ketoprofen–ABP	5.822	7.082	6.589	6.407	5.618

a chromatographic software CSW v.1.7 for Windows (Data Apex s.r.o., Prague, Czech Republic).

Analyses were performed on a 5 μm SUPELCO Discovery C18 125 mm × 4 mm i.d. column (Sigma–Aldrich) with pre-column SupelGuard 20 mm × 4 mm i.d., 5 μm (Sigma–Aldrich). The optimal mobile phase for separation of KP, MP, PP, ABP and CPA was a mixture of acetonitril, water and phosphate buffer pH 3.5 (40:58:2, v/v/v). Mobile phase was degassed before application by means of helium. The finally selected and optimised conditions were as follows: injection volume 10 μl, the mobile phase isocratically pumped at a flow rate 1.0 ml min^{-1} at ambient temperature and the detection wavelength 233 nm.

2.3. Sample preparation

An accurately weighed portion of pharmaceutical gel corresponding to 12.5 mg of ketoprofen (about 0.5 g) was transferred into a 50 ml centrifuge tube and supplemented with 20.00 ml of internal standard (10 μg ml^{-1} solution of ethylparaben in acetonitril). The mixture was placed into the ultrasonic bath for 10 min and then centrifuged at 1300 × *g* for 15 min. A volume of 10 μl of supernatant was analysed by HPLC.

Identification of peaks in the gel samples was based on the comparison of retention times of compounds in standard solutions. Peak identity was confirmed by UV–vis spectra.

3. Results and discussion

3.1. Method development and optimisation

In the beginning, the main criteria for developing a successful HPLC determination of ketoprofen, preservatives and both degradation products in a topical gel were established: the method should be stability indicating, free of interference from excipients and straightforward enough for routine use in quality control laboratory.

There is no any method for simultaneous determination of all five compounds in the literature. The British Pharmacopoeia 1993, Addendum 1995 specifies HPLC method for HPLC determination of ketoprofen and its degradation products using a UV detection (λ = 233 nm) and mobile phase containing: phosphate buffer (pH 3.5):acetonitrile:water (2:43:55, v/v/v). The wavelength for the detection of low concentration of degradation products is optimal, but ketoprofen was not separated enough from propylparaben. In order to improve the separation, several experiments were

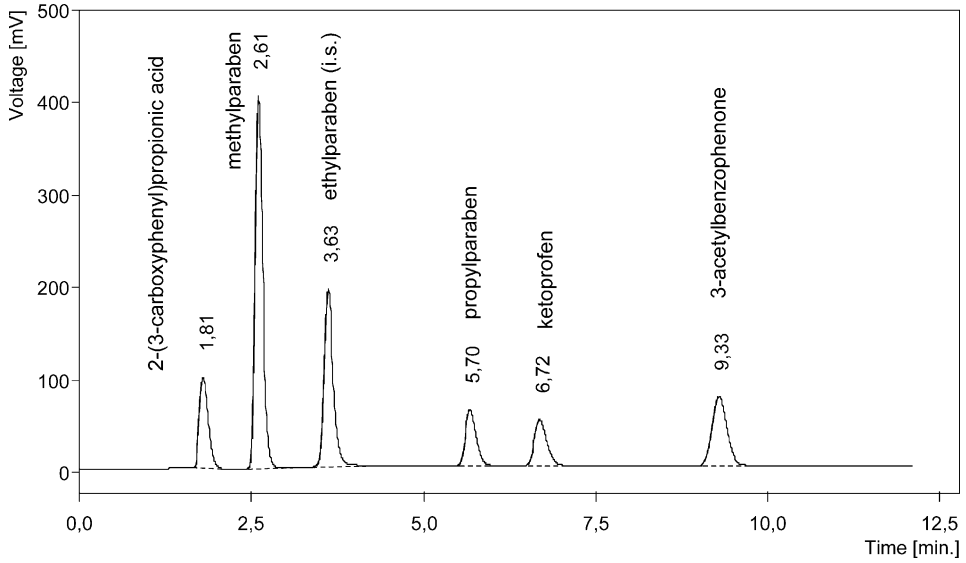

Fig. 2. Chromatogram of separation of standard solution for all compounds.

Table 2
Method validation results

Validation step	Parameter	KP[a]	MP[b]	PP[c]	ABP[d]	CPA[e]	Criteria
Repeatability	R.S.D.[f]	0.22%	0.37%	0.34%	0.28%	0.28%	$X < 2\%$
Method precision	R.S.D.[g]	1.20%	1.72%	1.83%	1.19%	1.89%	$X < 2\%$
Accuracy	Spike recovery[g]	101.3%	102.0%	102.6%	102.3%	102.0%	$X = 100 \pm 3\%$
	Recovery R.S.D.[g]	0.40%	0.31%	1.06%	0.47%	0.49%	$X < 2\%$
Linearity ($n = 6$)[h]	Correlation coefficient	0.9989	0.9987	0.9993	0.9996	0.9995	$X > 0.990$
Sample stability[i]	Percent change in response factors	1.20%	0.88%	0.53%	1.68%	1.96%	$X < 2\%$

[a] Ketoprofen.
[b] Methylparaben.
[c] Propylparaben.
[d] Degradation product: 3 acetylbenzophenone.
[e] Degradation product: 2-(3-carboxyphenyů) propionic acid.
[f] Six injections.
[g] Three preparations each, two injections of each preparation.
[h] At 20, 50, 80, 100, 120, 150% levels for KP, MP and PP and from 0.025 mg 100 ml^{-1} to 0.25 mg 100 ml^{-1} for degradation products.
[i] 2-Day stability data for all compounds.

performed; the final optimal mobile phase was: phosphate buffer (pH 3.5):acetonitrile:water 2:40:58 (v/v/v).

Firstly, flurbiprofen as internal standard was tested, giving a relatively high retention time of 13.3 min. Therefore, ethylparaben was tested with successful separation from other compounds, having the retention time about 3.6 min. After some preliminary experiments, ethylparaben was chosen for further experiments.

Different flow rates between 0.6 ml min^{-1} and 2.0 ml min^{-1} were tested. All six compounds were always well separated. Flow rate of 1.0 ml min^{-1} was chosen as optimal, since the peaks resolution was within then limits of acceptance (Table 1). The final composition of the mobile phase was: phosphate buffer (pH 3.5):acetonitrile:water 2:40:58 (v/v/v). Using a 5 μm packing of the column SUPELCO Discovery C18 and decreasing the flow rate to 1.0 ml min^{-1}, the total time of analysis for all compounds was less than 10 min. Fig. 2 shows a chromatogram of standard solutions of ketoprofen, preservatives, degradation products ABP and CPA and ethylparaben as internal standard.

3.2. Analytical parameters and validation

The optimised method was validated by a standard procedure to evaluate if adequate accuracy, precision, selectivity and linearity had been achieved. Accuracy was determined using spiked placebo solutions, three preparations each, two injections of each preparation. Relative standard deviation (R.S.D.) values were calculated for repeated standard injections (system precision) as well as repeated injections of multiple sample preparations (method precision). Linearity was determined in the 20–150% range at six different concentrations. Short-term stability of standards was evaluated by comparison of response factors of fresh and stored standards. Visual inspection of chromatograms of standards and placebo solutions was conducted to ensure selectivity. The method validation results obtained under the final conditions are shown in Table 2. The method meets all common requirements for accuracy, precision and linearity.

3.3. Analysis of degradation products

In the pharmaceutical formulation containing ketoprofen, a small amount of degradation products ABP and CPA can be found after a long-term storage. For the determination of the actual amount of the degradation products, it is necessary to realize that it occurs in a very low concentration level in comparison with the KP (about 100–1000 lower). This is also the reason why methods for determination of active compounds and methods for determination of degradation products are generally different. In our case, finally we found sufficient separation of all compounds together and therefore we could include the whole procedure into one step. Under the optimised conditions, the selectivity of the determination is sufficient and the retention time for ABP and CPA is far enough from the other compounds presented in the sample. Limit of detection (LOD) for 10 μl injection of ABP standard (at signal to noise S/N = 3) was 0.014 μg ml^{-1} (0.00224% of KP); limit of quantification (LOQ) for ABP was 0.046 μg ml^{-1} (0.00736% of KP); LOD for CPA was 0.040 μg ml^{-1} (0.0064% of KP), LOQ 0.135 μg ml^{-1} (0.0216% of KP). The values of amounts of degradation products after 6 months (Fig. 3) were 0.0076% of KP for ABP and 0.102% of KP for CPA. The values are higher than LOQ of both degradation products.

3.4. Determination in pharmaceutical product

The chromatogram in Fig. 3 was obtained using the described LC method with a sample of topical Ketoprofen gel 2.5% after a long-term stability test (stored for 6 months in the original packaging at 25 ± 2 °C and relative humidity 60 ± 5%). All compounds presented in the sample, ketoprofen, both preservatives, degradation products and internal standard, are clearly separated. The average determined amounts

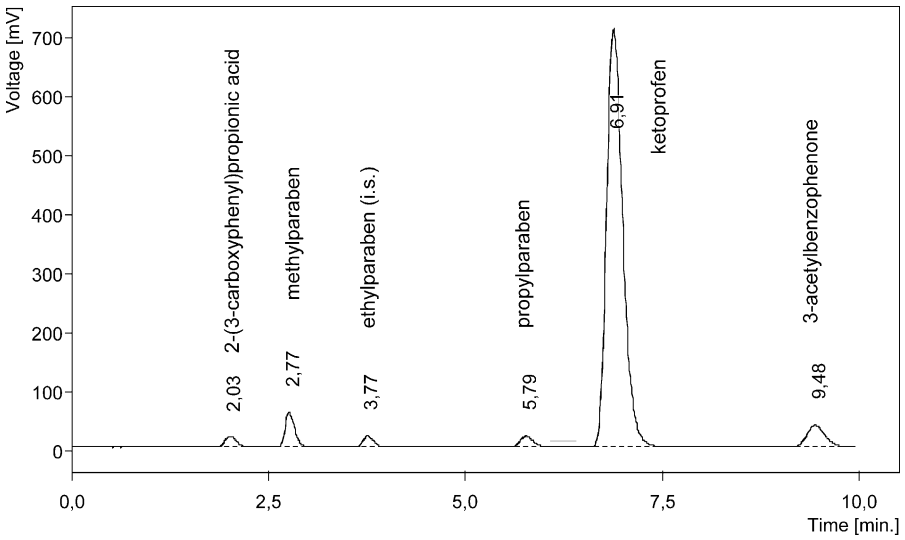

Fig. 3. Chromatogram of all compounds in a topical gel after a 6-months stability test.

of the KP, MP and PP in Ketoprofen gel 2.5% were 98.7 ± 0.9%, 99.0 ± 1.0% and 102.1 ± 0.6% of the labelled amount, respectively.

4. Conclusion

The LC method with UV spectrophotometric detection on a SUPELCO Discovery C18 column was developed for the determination of ketoprofen, 3-acetylbenzophenone, 2-(3-carboxyphenyl) propionic acid, methylparaben and propylparaben in a topical gel, using ethylparaben as an internal standard.

The total analysis time was less than 10 min. The method has been validated; the results obtained were precise and accurate and the limits of detection of degradation products were sufficiently low. The method can be used for routine analysis (batch analysis and stability tests) of compounds in pharmaceutical products containing the active compound ketoprofen, preservatives methylparaben and propylparaben and two degradation products of the active compound.

The proposed method was successfully applied for the identification, quantitative analysis and stability tests of all major compounds in the topical gel—Ketoprofen gel 2.5%.

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