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Original Article

HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF KETOPROFEN AND PRESERVATIVES IN GEL FORMULATION

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ABSTRACT

Objective: The aim of presented study was to develop a high-performance liquid chromatography (HPLC) method with UV detection for simultaneous determination of ketoprofen, methyl paraben and propyl paraben in a gel formulation.

Methods: The chromatography was carried out on a C_{18} (250 mm x 4.6 mm, 10 µm) column with methanol, acetonitrile and 1.5 % sodium acetate solution (15:35:50 v/v/v) as mobile phase, at a flow rate of 1.0 ml/min, with detection at 240 nm.

Results: Under these chromatographic conditions, the obtained retention times were approximately 2.41 min for methyl paraben, 3.33 min for propyl paraben and 7.41 min for ketoprofen. Analytical parameters specificity, linearity, accuracy, precision and robustness were determined by validation procedure and found to be satisfactory.

Conclusion: Overall, the proposed method was found to be simple, rapid, precise and accurate for quality control of ketoprofen in the presence of preservatives in gel formulation.

Keywords: Liquid chromatography, Ketoprofen, Methyl paraben, Propyl paraben, Validation, Gel formulation.

INTRODUCTION

Ketoprofen (KET) is a non-steroidal anti-inflammatory drug (NSAID) with well established analgesic and antipyretic properties used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and gout [1]. Although ketoprofen is poorly water soluble it is rapidly absorbed, metabolized and excreted, it causes some gastrointestinal complaints such as nausea, dyspepsia, diarrhea, constipation and some renal side effects like other NSAIDs. Topically applied NSAIDSs have several advantages in comparison with their systemically administrated counterparts. These drugs are simple for application and deliver high drug concentrations locally into affected tissues with limited side effects at the application site for prolonged periods [2].

Several types of analytical procedures have been proposed for the analysis of ketoprofen in pharmaceutical formulations. The procedures include capillary zone electrophoresis [3], UV-spectrophotometry [4, 5], high-performance liquid chromatography [6,7], flow injection technique with hemiluminiscence [8], flow injection with UV-detection [9], polarography [10], potentiometry [11], and quantitative Fourier transformation infrared spectrophotometry [12]. European Pharmacopoeia recommends acid-base titration for analysis of ketoprofen in substance, UV-spectrophotometry for its determination in capsules as well as liquid chromatography for assay in gel [13].

Methyl 4-hydroxybenzoate (methyl paraben) and propyl 4-hydroxybenzoate (propyl paraben) are well-known preservatives used primarily for their bactericidal and fungicidal properties. Parabens are a group of alkyl esters of p-hydroxybenzoic acid, having a low toxicity profile and a long history of use. It readily absorb from the gastrointestinal tract or through the skin [14]. The use of methyl paraben is toxic at higher concentrations due to estrogenic effect [15]. The estrogenic activity of parabens increases with the length of the alkyl group and it is well known that propyl paraben is estrogenic to a certain degree as well [16].

The determination of these substances in pharmaceuticals and cosmetics is important in quality control, especially considering the numerous reports of allergic reactions caused by preservatives. Some methods of analysis of investigated parabens either alone or in some other formulations are available including HPLC [17-25], solid phase extraction HPLC [26, 27], HPLC-MS [28].

Simultaneous determination of both parabens and ketoprofen is reported by several authors [29-33].

The aim of this paper was to develop a specific, precise and accurate chromatographic method able to be applied in quality control for the determination of ketoprofen in gel formulation in the presence of both preservatives methyl- and propyl parabens.

MATERIALS AND METHODS

Chemicals and Reagents

Ketoprofen, methyl- and propyl parabens were purchased from Sigma-Aldrich (Germany) as standards. A ketoprofen gel containing 2.5 % w/w active compound, 1 % w/w methyl paraben and 0.1 % w/w propyl paraben was obtained commercially. LC-grade methanol and acetonitrile were supplied from Merck (Germany). All other chemical reagents were of analytical grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector, column oven CTO-10A, SPD-M10A fixed wavelength detector and communication bus module CBM-10A. A LiChrosorb C_{18}, 250 mm x 4.6 mm, 10 µm column was used as a stationary phase. The components were separated isocratically with a mobile phase consisting of 15 volumes methanol, 35 volumes acetonitrile and 1.5 % sodium acetate solution at a flow rate of 1.0 ml/min. The analysis was carried out at an ambient temperature and injection volume was 20 µl. The UV detector was set at 240 nm.

Preparation of reference solutions

Reference solution (a): The solution was prepared by dissolving 20.0 mg of accurately weighed methyl paraben in methanol in a 50.0 ml volumetric flask (C=400 µg/ml).

Reference solution (b): The solution was prepared by dissolving 20.0 mg of accurately weighed propyl paraben in methanol in a 100.0 ml volumetric flask (C=200 µg/ml).

Working reference solution: The solution was prepared by dissolving of accurately weighed 25.0 mg ketoprofen in the first step and in the second step by diluting 25.0 ml of reference solution (a) and 5.0 ml of reference solution (b) with methanol into a 50.0 ml volumetric flask. The concentrations of the investigated compounds in working reference solution were as follows: ketoprofen – 500 µg/ml, methyl paraben – 200 µg/ml and propyl paraben – 20 µg/ml, respectively.

Sample preparation

2 ml (accurately measured) of ketoprofen gel corresponding to 50 mg ketoprofen, 20 mg methyl paraben and 2 mg propyl paraben were transferred to a 20 ml volumetric flask. Ten ml of methanol were added and the flask was placed in an ultrasonic bath for 20 min. After cooling at the room temperature, the flask was filled with methanol to the volume mark, and the sample was centrifuged at 2000 rpm for 10 min. Five ml of the clear centrifugate was transferred to a 25 ml volumetric flask, and filled with mobile phase to the volume mark. After filtration through a 0.45 mm membrane filter, an aliquot of the sample solution was injected into the HPLC column.

Validation procedure

The analytical method developed was validated with respect to specificity, precision, accuracy, robustness and sensitivity.

Specificity

The ability of an analytical method to unequivocally assess the analyte in the presence of other components can be demonstrated by evaluating specificity. The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution.

Linearity and range

The linearity of the method was determined at six concentration levels ranging from 125 to 1000 µg/ml for ketoprofen, from 50 to 400 µg/ml for methylparaben and from 5 to 40 µg/ml for propyl paraben. The calibration curves were constructed by plotting peak areas versus concentrations of investigated compounds, and the regression equations were calculated. Each response was the average of three determinations.

Precision

Intraday precision (repeatability) was calculated using two concentrations of KET (250, 500 µg/ml), methyl paraben (100, 200 µg/ml) and propyl paraben (10, 20 µg/ml) in triplicate using proposed methods. The inter day precision (reproducibility) was repeated three times on three different days for analysis of two different concentration (250:100:10, 500:200:20 µg/ml) for analyzed drugs.

Accuracy

Accuracy of the method was evaluated by standard addition technique, which was performed by addition of known amounts of pure ketoprofen and both parabens to known concentrations of gel and analysed by proposed methods in triplicate.

Robustness

The robustness of the method is a measure of the capacity to remain unaffected by small variations in method parameters and provides indication of its reliability during normal usage. Robustness of the analytical procedure was studied by deliberately varying parameters like analytical wavelength (±2 nm) and flow rate (±0.2 ml/min). Column-to-column reproducibility was also checked by using a C18 column of different make (Nucleosil) with same dimension.

RESULTS AND DISCUSSION

Fig. 1, showed that under the experimental chromatographic conditions ketoprofen, methyl paraben and propyl paraben were completely separated one from each other. In the chromatogram of blank solution there are no interfering peaks at the retention times of the investigated peaks. This indicated that the method is selective and can be used for identification and simultaneous quantification of ketoprofen in the presence of preservatives in topical gel formulation.

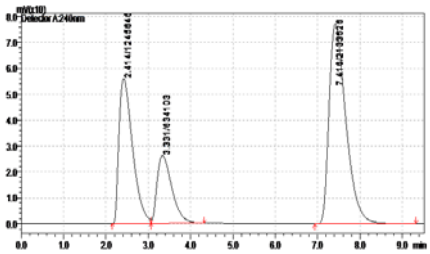

Fig. 1: Chromatogram of working standards: methyl paraben, propyl paraben and ketoprofen

Retention times, number of theoretical plates and tailing factors obtained by using of the HPLC method were listed in Table 1.

Table 1: Chromatographic data from HPLC method (system-suitability test)

Parameter	methyl paraben	propyl paraben	ketoprofen
Retention time (min)	2.41	3.33	7.41
Tailing factor	0.91	0.89	0.90
Theoretical plates	2578	1354	1523

Calibration and linearity

We prepared a series of 5 calibration solutions with a concentration range shown in Table 2. It was found that response (peak area) was proportional to concentration over the ranges tested with correlation coefficients greater than 0.9996. Calibration plot data slope (a), intercept (b), and correlation coefficient (r) were listed in Table 2.

Table 2: Validation data for the calibration plots

Compounds	methyl paraben	propyl paraben	ketoprofen
Concentration range (µg/ml)	50-400	5-40	125-1000
Slope	2456.8	34111.7	47854.4
Intercept	678.1	-946.4	7845.1
Correlation coefficient (r)	0.9996	0.9999	0.9998

Precision

The values of % RSD (Table 3) for KET, MP and PP were found to be in the range from 0.32 to 0.87 indicating good repeatability and reproducibility of the analytical procedure.

Robustness

It was found that the elution order and resolution for both components were not significantly affected by small variation of the conditions. Results from study of the robustness of the method were listed in Table 5.

Limit of detection and limit of quantification

The limits of quantification (LOQ) and limit of detection (LOD) were evaluated based on signal-to-noise ratios by serial dilution of working reference solution. The LOQs for ketoprofen, methyl paraben and propyl paraben were found to be 1.0, 0.8 and 0.5 µg/ml respectively; the LODs – 0.2, 0.1 and 0.05 µg/ml, respectively.

Table 3: Precision of the method

Precision	Amount taken (µg/ml)			% Mean*			% RSD		
	KET	MP	PP	KET	MP	PP	KET	MP	PP
Intra day	250	100	10	99.56	100.5	99.61	0.36	0.32	0.54
Intra day	500	200	20	100.1	100.2	100.9	0.65	0.61	0.61
Inter day	250	50	10	99.48	99.89	99.81	0.84	0.56	0.38
Inter day	500	150	20	99.75	99.52	99.62	0.78	0.87	0.42

**Mean of three determinations*

Results presented in Table 4 indicated good accuracy and showed no interference from tablet excipients.

Table 4: Recovery studies of ketoprofen and parabens

Compound	Amount taken (µg/ml)	Amount added (µg/ml)	Amount recovered ± SD* (µg/ml)	% RSD
		125.0	625.7±1.50	0.24
ketoprofen	500.0	250.0	749.8±1.98	0.26
		375.0	873.8±3.41	0.39
		50.00	248.4±0.95	0.38
methyl paraben	200.0	100.0	297.1±0.98	0.33
		150.0	348.7±1.15	0.33
		5.00	24.81±0.25	1.01
propyl paraben	20.0	10.00	29.68±0.31	1.04
		15.0	35.87±0.28	0.78

*Average value of three determinations, RSD is relative standard deviation

Table 5: Robustness parameters of LC method

Factor	Level	ketoprofen		methyl paraben		propyl paraben	
Analytical wavelength		**Assay, %**	**% RSD**	**Assay, %**	**% RSD**	**Assay, %**	**% RSD**
238	-2	99.84	0.54	100.0	0.65	98.8	0.98
240	0	100.5	0.48	99.65	0.93	99.6	1.05
242	+2	99.21	0.97	99.48	0.92	99.1	0.87
Flow rate of mobile phase							
0.8	-0.2	98.79	1.24	98.70	0.64	98.2	0.56
1.0	0	99.36	0.98	101.0	0.89	99.6	0.85
1.2	+0.2	99.25	0.97	99.82	0.97	99.8	1.21
Stationary phase							
Nucleosil C 18	-	101.2	1.08	99.81	0.91	99.1	1.04
LiChrosorb C18	-	99.89	0.84	99.55	0.98	99.6	0.95

CONCLUSION

The validated RP-LC method developed here proved to be simple, specific, accurate, precise, sensitive and robust. It can successfully used for routine analysis of ketoprofen in the presence of described preservatives in gel formulation without any interference from common excipients.

CONFLICT OF INTERESTS

Declared None

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