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Stability Indicating LC Method for the Determination of Ketoprofen in Presence of its Impurity.

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ABSTRACT

The analytical chemistry has to be a part of the progress of the researches on the contamination of the water by medicines; she has to establish processes of validated analyses, for the detection and the dosage of diverse pharmaceutical substances. On this matter the proposed analysis concerns the detection and the dosage of the ketoprofen within all products of damages. A high performance liquid chromatographic method for the determination of ketoprofen in the presence of one related impurity and its degradation products is described. The method is based on the use of an C_{18} phase column (SUPERCOSIL ^{TM} LC-18-DB) and a mobile phase of acetonitrile-potassium phosphate acid (0.05 M, pH 4.5) - water (43: 2: 55, v/v/v)with 0,3 millimoles of Octyl Sulfate of sodium (OSS),all at pH 4,5. All peaks are eluted in <15 min. The method was demonstrated to be precise, accurate and specific. Degradation study showed that the drug is stable in basic medium while it degrades under oxidative conditions and acidic medium. The results indicated that the proposed method could be used in a stability assay.
Keywords: Pharmaceuticals; ketoprofen; Liquid chromatography; Degradation products

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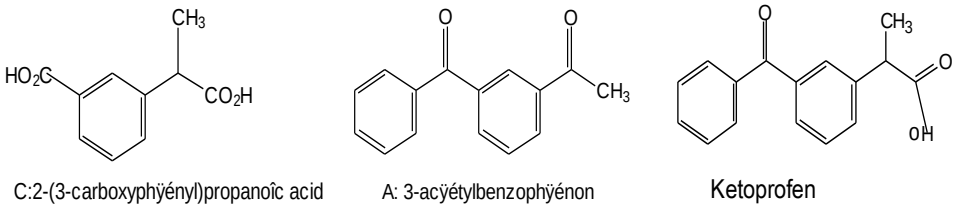
INTRODUCTION

Ketoprofen, (RS)-2-(3-benzalphenyl) propionic acid, is an anti-inflammatory non steroidien from the propionic class [1]. Its main therapeutic applications are for rumathoid diseases for human and veterinary medicines [1-9]. its action of mechanism is to stop the cyclogenase and lipoxygenase formation [10-13].

A literature survey revealed few analytical methods for determination of Ketoprofen. Furthermore, its estimation in biological samples has been described by gas chromatography [1], [14], [15] and [16] and high performance liquid chromatography [17-31]. Differential-pulse polarography [32-35], and an high performance liquid chromatography couplet with mass spectrum[36,37] methods are reported for its determination in pharmaceuticals, but so far no data on specificity of the methods with respect to impurities is available.The purpose of this work was to develop a procedure for the quantification of kétoprofène and its separation, mainly, from its related substances. In addition a forced degradation studies of ketoprofen were performed to define its degradation process and to provide an indication of the stability-indicating and specificity of the method. The considered impurities are reported in Fig. 1.



C:2-(3-carboxyphÿényl)propanoîc acid A: 3-acÿétylbenzophÿénon Ketoprofen

Figure 1: Chemical structures of ketoprofen and its degradations products (C–A)

RESULTS AND DISCUSSION

Separation studies

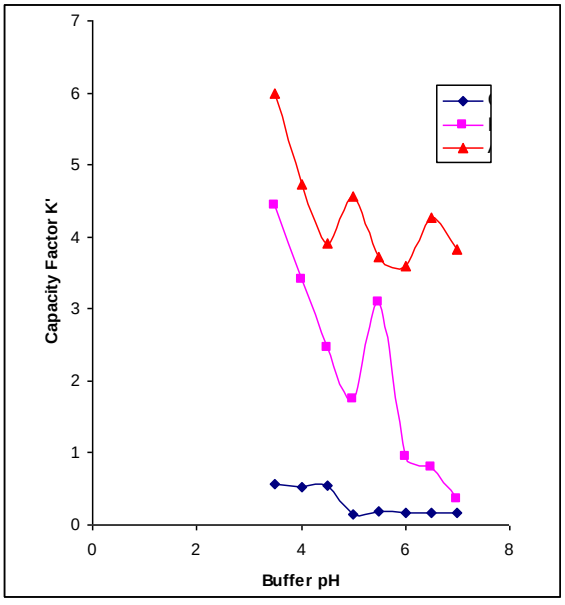


Figure 2: Effect of buffer pH on capacity factor of ketoprofen and its impurities.

To optimize the separation of the different compounds under isocratic conditions, the effect of both buffer pH and organic modifier were investigated. Fig. 2 shows the influence of pH on the capacity factor of each of the examined compounds with mobile phase of acetonitrile- potassium phosphate acid (0.05 M, pH 4.5) - water

(43: 2: 55, v/v/v)with 0,3 millimoles of Octyl Sulfate of sodium (OSS),all at ph 6. This graphic representation shows that an increase of pH led, as expected, to the decrease of the retention time of ketoprofen, while, little decrease of the retention was obtained for A and C, which behave as acids [47].

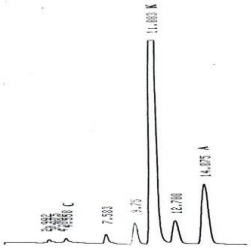


Figure 3: LC chromatogram of ketoprofen and its suggested impurities (A and C) after 12h of degradation by hydrogen peroxide.

However, this change in the mobile phase pH did not lead to a complete separation. Nevertheless, it appears that better result was obtained at pH 4.5. On the other hand, a second mobile phase consisting on of acetonitril- phosphate buffer - water (43: 2: 55, v/v/v) [17], led to a decrease of the analysis time without improvement of separation. Therefore, the addition of an optimum concentration of phosphate buffer to this last mobile phase with decreasing the one of acetonitril (41:4:55) was tried and a good result was obtained. A third mobile phase consisting of the second one with addition of 0,3millimoles of SDS (sulphate dedocyl of sodium) was tried too and also a good result was obtained. The selected eluent yielding appropriate peak separation was of acetonitril- potassium phosphate acid (0.05 M, pH 4.5) - water (43: 2: 55, v/v/v) with 0,3 millimoles of Octyl Sulfate of sodium (OSS),all at pH 4,5.. The chromatogram of the tree compounds known and three compounds unknown obtained from the most degradation solution obtained with hydrogen peroxide with was heated for 12H under the suggested conditions is depicted in Fig. 3.

Linearity

Three 5-point calibration curves, performed on three different days, were plotted as the peak area versus concentration. The results of regression analysis parameters summarized in Table 1 showed that the method was linear, with a correlation coefficient greater than 0.999. The mean slope had a low R.S.D. (<2%) and the mean intercept was not significantly different from the theoretical value of zero.

Table 1: Statistical study of linearity of ketoprofen

Range of concentration (µg ml^{-1})	20–60		
Slope	657.69	(R.S.D.=0.456 < 2%)	
Intercept	928.6		
Correlation coefficient	0.999		
		Theoretical values	Conclusion
Comparison of intercept with 0 (*t*-test)	2.158	$t_{(0.05;\ 13)}$ = 2.160	NS
Homogeneity of variance (test of Cochran)	0.391	$C_{(0.05;\ 5;\ 2)}$ = 0.68	NS
Existence of a significant slope (test of fisher)	4207.12	$F_{(0.05;\ 1;\ 13)}$ = 4.67	HS
Validity of adjustment (test of Fisher)	3.192	$F_{(0.05;\ 3;\ 10)}$ = 3.7	NS

NS: not significant. HS: highly significant.

Precision and accuracy

Repeatability was assessed by injecting a standard solution of ketoprofen and one of the product sample at three different levels six times in the same day. Between-days precision was evaluated by 18 determinations of ketoprofen standard solution, at three different concentrations for three consecutive days (six determinations per day for each concentration). The obtained R.S.D. values for the intra-day and inter-day were less than 2% (Table 2) indicating a satisfactory result. The accuracy of the method was demonstrated by recovery experiments, using the standard addition technique. Different levels of standard ketoprofen were added to pre-analyzed tablets. The determination was carried out using three replicates at each level. Satisfactory recoveries (Table 3) were obtained, and no significant differences were observed between the amount of ketoprofen added and the amount found, which indicated the accuracy of the method.

Table 2: Precision of the method

	Reproducibility (*n* = 18 within 3 days)					
Concentration of ketoprofen (µg ml^{-1})	20	40	60	20	40	60
Found mean	19.0040	36.0642	57.8781	19.7430	38.2132	58.7646
R.S.D. (%)	2.2317	2.2035	1.8624	3.5550	3.8914	2.6117

Table 3: Accuracy/recovery of ketoprofen in synthetic preparations

Amount added (µg ml^{-1})	**Amount found (µg ml^{-1})**	**Recovery (%)**	**R.S.D. (%) (*n* = 3)**
20	19.755	98.78	1.6195
30	30.247	101.82	1.2297
40	40.035	100.09	0.1590
50	50.675	101.35	0.2093
60	59.625	99.38	0.8420

LOD and LOQ

The detection limit, based on a signal to noise ratio of 3:1 and 20 µl injections, was found to be 2.67 ng ml^{-1}. The quantisation limit with a signal to noise of 10:1 and 20 µl injections was found to be 4 ng ml^{-1}.

Degradation studies

The resulting chromatograms for a standard mixture with those of ketoprofen solutions obtained under stressed conditions for 4h are shown in Fig. 4. The degradation products are well resolved for degradation peaks were identified by their retention time, which were identical to the reference substances available in our laboratory (Table4), ketoprofen and degradation products do not interfere.

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Table 4: Retention time of ketoprofen ,A and C under stress conditions and detected by the proposed method :

$T_{RX} \pm$ SD; RSD% witch X = C ; K ;A

Conditions	pH	Retention time ± SD ;RSD% of		
		C	Ketoprofen	A
HCl 2N	4,5±0,01	2,950±0,0024; 07596%	8,6300±0,1557; 2,9786%	12,6007±0,0670; 0,4927%
NaOH 2N	4,5±0,01	4,1023±0,0670; 1,6327%	10,2690±0,0637; 0,6199%	13,7337±0,0656; 0,4778%
H_2O_2 30%	4,5±0,01	3,7967±0,0664; 1,7496%	10,2863±0,1390; 1,3513%	13,7323±0,1549; 1,1283%

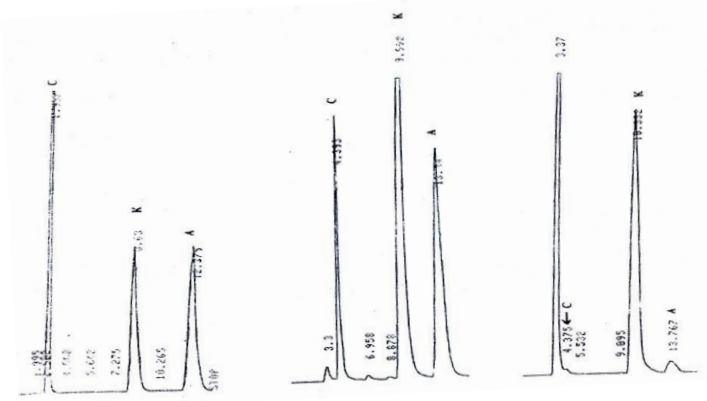

Figure 4: LC chromatograms from ketoprofen degradation study. (A) acidic degradation; (B) basic degradation; (C) hydrogen peroxide degradation.

The chromatogram obtained after acidic degradation generated tree major products. The tree peaks, corresponding to ketoprofen (t_R =8,63min), one of them (t_R = 2,937min), was identified as the 2-(3-carboxyphenyl) propanoîc acid :C and the therd peak (t_R =12,375min) could attributed to the 3-acetylbenzophenone :A. This degradation is favoured by the formation of carbocation formed by the presence of ketone functionality.

The solution obtained from refluxed ketoprofen in sodium hydroxide led to a chromatogram with tree majors products (t_R =9,992min), which corresponds to ketoprofen, (t_R = 4,393min), which corresponds to compound C and the third one (t_R =13,34min) could attributed to the degradation product A. This result indicates that ketoprofen, undergoes a nucleophilic substitution of the fluorine atom by the hydroxyl group. As well known, this reaction is favoured by the presence of ketone functionality in the Para position [48].The degradation performed by hydrogen peroxide generated only one peak.(t_R =10,332min) which corresponding to the ketoprofen This result indicates a good stability of this compound in hydrogen peroxide. But also the compounds C and A were identified respectively at (t_R = 4,375min) and(t_R =13,767min).

Assay of ketoprofen

The proposed method was applied to the determination of ketoprofen in tablets formulation (Felden 50 mg of ketoprofen). The mean average (three replicates) was found to be 50.675 mg corresponding to a mean recovery of 101, 35% with an R.S.D. of 0.2093%. This result was in good agreement with the label value. On the other hand, it should be pointed out that the chromatogram of the solution of excipients is absolutely free of any peak indicating thus that no interference from the excipients is encountered.

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EXPERIMENTAL

Samplers

Ketoprofen, (RS)-2-(3-benzalphenyl) propionic acid, 3-acetylbenzophynon: A and the 2-(3-carboxyphenyl)propanoîc acid: C, were kindly provided by Aventis pharma SA (Megrine, Tunisia). The pharmaceutical formulation used in this study was Profaned tablets and also provided by Aventis pharma SA (Megrine, Tunisia).

Reagents

Methanol, acetonitril were of HPLC grade, from Fisher chemicals (UK). Hydrogen peroxide, phosphoric acid, sodium hydroxide and potassium phosphate acid were purchased from Prolabo (France).OSS (octyl sulphate of sodium) and SDS (sulphate dedocyl of sodium) from LNCM (National laboratory of controls of drugs, Tunisie). Water was bidistileted. All solid and liquid reagents were reagent grade.

Apparatus

A Shimadzu LC system (Kyoto, Japan) composed of an LC-10AT VP pump equipped with a 7725i Rheodyne (CA, USA) injector, an Perkin-Elmer LC 295 UV–vis detector and a C-R3A Chromopac Shimadzu integrator was used. The pH values were measured with a SCHOTT CG 825 pH meter.

Chromatographic conditions

The separation was performed on a 25 cm × 4.6 mm C_{18} SUPELCOSIL^{TM} LC-18-DB column (Supelco, Bellefonte, PA, USA). The flow rate was 1.0 ml min^{-1}. The injection volume was 20 µl. The detection wavelength was set at 233 nm. The mobile phase consisted of acetonitrile- potassium phosphate acid (0.05 M, pH 4.5) -water (43: 2: 55, v/v/v) with 0,3 millimoles of Octyl Sulfate of sodium (OSS),all at pH 4,5.

To determine the effect of buffer pH on the separation of ketoprofen, and its suggested degradation products, six different mobile phases were prepared at Ph of 4.5, 5, 5.5, 6, 6.5 and 7. For the estimation of the capacity factor, a solution of sodium nitrate (10 µg ml^{-1}) was used as a non retained substance in order to determine the void retention.

Preparation of sample solutions

Quantities between 9 and 10, 3 mg of ketoprofen and the examined products were dissolved separately in 10 ml of the mobile phase and were labelled as stock solutions. For the determination of the retention time of the different compounds, reference solutions were separately prepared by diluting 1 ml of each stock solution to 10 ml with the mobile phase. To optimize and evaluate the separation of all the analysts from each other, a mixture of the three substances containing 1 ml from each stock solution was prepared in a 10 ml volumetric flask and was diluted to volume with the mobile phase.

Calibration solutions and sample assay

In order to check the response linearity of the method, five calibration solutions over the range of the desired concentrations were prepared by appropriate dilutions of the calibration stock solution of ketoprofen (1000 µg ml^{-1}). The mobile phase was used as solvent for all preparations. Tablets, 20 units were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 50 mg of ketoprofen was transferred into a 50 ml volumetric flask and sonicated for 5 min with 50 ml of the mobile phase. The resulting suspension was filtered through 0.22 µm membrane filter. A suitable aliquot of this filtrate was diluted with the mobile phase in order to obtain a final concentration of 1000 µg ml^{-1}. A 20 µl of the obtained solution was chromatographed. The determination of sample solution was carried out by using the calibration curve. The injection sequence included a

blank solution (the mobile phase), the tree calibration standard solutions, a solution of excipients and finally the sample solution.

Validation parameters

Linearity, accuracy and precision were determined according to the statistical method of validation described previously [38-46]. The percentage recovery of the ketoprofen was computed from the regression equation.

Ketoprofen degradations

Fifty milligrams of ketoprofen was mixed separately in 50 ml of 2N HCl, 2N NaOH and 30V H_2O_2. The mixtures, obtained with either hydrochloric acid or sodium hydroxide were refluxed for 4 h and 12H, while, the one obtained with hydrogen peroxide was heated at 80 °C for 4 h and also at 12H.Each resulting solution was cooled at room temperature and filtered. An aliquot of 1 ml was neutralized when it was necessary and diluted with methanol to 5 ml. All these solutions were analyzed using LC.

CONCLUSION

A simple and rapid stability-indicating LC method has been developed for the determination of ketoprofen in the presence of its impurity and degradation products. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The method is reliable and convenient for routine control and stability assays of ketoprofen in both raw material and tablets.

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