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A validated method development for ketoprofen by a flow-injection analysis with UV-detection and its application to pharmaceutical formulations

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Abstract

A simple, sensitive and fast flow-injection analysis method with UV-detection method was developed for the determination of ketoprofen in pharmaceuticals. The standard and sample solutions were dissolved in a 10% ethanol which was suitable for this study. A flow-rate of 0.6 ml min^{-1} was used and the analyte was monitored at 260 nm. Variables such as concentrations, flow rate of reagents and other flow injection parameters were optimized to produce the most sensitive and reproducible results. Linear calibration curves were obtained in the range of 1.6×10^{-6} and 1.7×10^{-4} M. Limit of detection and limit of quantification were 1.7×10^{-6} M ($S/N = 3.3$) and 5.3×10^{-6} M ($S/N = 10$), respectively. The method was applied successfully to the analysis of ketoprofen pharmaceutical tablets. The recoveries were 102.75% for peak area and 98.42% for peak height. The proposed method is fast, precise, sensitive and easy to use for the determination of ketoprofen in pharmaceuticals.
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1. Introduction

Ketoprofen (KET, Fig. 1) (3-benzoyl-α-methylbenzeneacetic acid) is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. It has potent inhibitory effects on prostaglandin synthesis. It is commonly used in the treatment of rheumatoid arthritis and osteoarthritis. Therapeutic doses of KET have proven to be as effective as those of the other commonly used NSAID [1,2].

Several methods have been reported for the determination of KET by HPLC in plasma and tablets [3], in blood [4] its enantiomers in plasma and urine [5]. Besides, spectrophotometry [6], polarography [7], capillary electrophoresis CE [8] have been employed. Additionally, a capillary zone electrophoresis (CZE) method for the analysis of enantiomers of KET in pharmaceuticals has been reported [9].

Flow-injection analysis is a useful analytical technique since it is fast, cheap, accurate and precise, and had very wide applications in many areas. This study describes a simple, rapid, sensitive and practical method for the determination of KET in pharmaceutical formulations using flow injection analysis. The optimum parameters were investigated and method validation studies were performed. The proposed method proved suitable for the routine quality control analysis of pharmaceutical formulations containing KET.

2. Experimental

2.1. Chemicals

The standard KET was supplied from Nobel Ilaç San. ve Tic. A.S. (Istanbul,Turkey). Other chemicals were of

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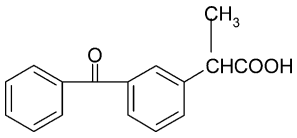

Fig. 1. The chemical structure of KET.

analytical grade and provided from Merck (Darmstadt, Germany). Distilled ethanol and distilled water was produced in our laboratory using completely glass apparatus. The commercial preparation of KET, Profenid® retard tablets (each containing 200 mg KET, Eczacibasi Rhône Poulenc, Istanbul, Turkey), was purchased from the local market.

2.2. Apparatus

Spectrophotometric studies were conducted using UV-2401 PC Spectrophotometer. (Shimadzu, Japan). The flow-injection analysis were conducted employing a model LC 6A pump and signals were detected by a model of SPD-10A UV-Vis detector and data were processed by a model of CR 7A integrator (Shimadzu, Japan). Standard solutions and samples were injected to a Rheodyne 20-μL loop injection port (Cotati, CA) by a 22-gauge injection needle. Mobile phase was always filtrated from a glass filter. Carrier solvent system was always sonicated with using an ultrasonic bath B-220 model (Branson, CA).

2.3. Procedures

2.3.1. Preparation of solutions

A standard stock solution 1×10^{-3} M of KET was prepared by dissolving the necessary amount of KET in 10 ml of ethanol and adding one to two drops of 1 M NaOH, then it was made up to 100 ml by addition of double distilled water. This stock solution was employed for the preparation of other dilutions. Mobile phase was an aqueous solution of ethanol (10%, v/v).

2.3.2. Flow-injection analysis

Flow-injection analysis was performed in a supporting solution consists of 10 percent ethanol. The signals were detected at 260 nm where monochromatic light is absorbed maximum. Standard and sample solutions were injected to a 20-μl fix volume of loop. The variation of flow–rate was examined in range of 0.1–2.5 ml min^{-1}.

2.3.3. Application of method to KET tablets

For the analysis of KET, ten Profenid® tablets (each containing 200 mg KET) were accurately weighed and grinded to fine powder. A sufficient amount of powdered tablet equivalent to the average weight of one tablet was accurately weighed, transferred to a 100-ml flask and ethanol was added to dissolve the active material. It was sonicated for 15 min and it was made up to 100 ml by double distilled water after adding one to two drops of 1 M NaOH. The solution was then centrifuged at $3000 \times g$ for 15 min. The supernatant was diluted with 10% ethanol and was injected.

3. Results and discussion

3.1. Optimization of the method

Method efficiency was examined at the beginning of this study. Solvent system must dissolve KET and it must be cheap, easily provided and must not be volatile. It was decided that 10% ethanol is very suitable solvent for these purpose.

Whole instrumental parameters were taken into account and they were examined during the examinations.

A 1.11×10^{-5} M KET solution was prepared, diluting with 10-percent ethanol from the stock solution. The spectrum of the solution was recorded in the wavelength range of 200–360 nm. A maximum appeared at 260 nm. Wong et al. [3] and Palylyk et al. [5] performed the analysis of KET at 254 and 275 nm, respectively. No interference was observed at the studied wavelength, i.e. 260 nm.

The effect of flow-rate variation was examined in the range of 0.1–2.5 ml min^{-1}. Very big peak areas or peak heights were exhibited in the small flow-rates. However, little peak areas or peak heights appeared when the flow-rate increased. The variation was similar to an exponential curve and it was fitting to (linear line) the function of reciprocal of signal. It fits to the equation of $[S(1/\text{area}) = 1.57 \times 10^{-6}\ \text{FR (min ml}^{-1}) + 0.65 \times 10^{-7}$; $r = 0.9997]$. Where; S is 1/area and FR is flow-rate.

Sharper and symmetrical peaks appeared in the range of 0.5–1.0 ml min^{-1}. Therefore, flow-rate of 0.6 ml min^{-1} was used at the rest of the study.

In summary, the analysis was carried out in a supporting solvent system consisting of 10 percent ethanol. The signals were measured at 260 nm, with the flow-rate of 0.6 ml min^{-1}. The signals of eight injections of 1.11×10^{-5} M KET in the mentioned conditions are shown in Fig. 2.

3.2. Validation of the method

Validation of the method was tested by examining the repeatability. A three sets of 5×10^{-5} M KET dilution was prepared and it was injected for three consecutive days. The output of the results as peak area and peak height were statistically evaluated presented in Table 1.

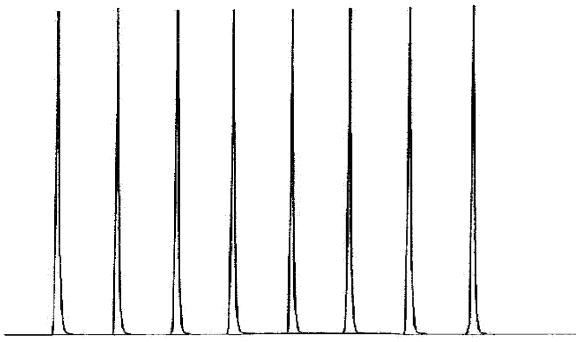

Fig. 2. The signals of eight injections of 1.11×10^{-5} M KET solution recorded at 260 nm with the flow-rate of 0.6 ml min^{-1}.

The results indicates very good repeatability. It also concluded that both peak area and peak height values may be used equivalently for quantitative studies.

3.3. Linearity

The linearity of the method was tested in the optimum determination conditions injecting in the concentration range of 5.5×10^{-6} and 2.8×10^{-5} M of KET. Three sets of dilutions in the above-mentioned concentrations were prepared and each set of dilutions were injected on consecutive days. These were the member of the intra-day analysis. Inter-day of analysis consisted the sum of the dilutions of sets. The results of linearity such as slope, intercept, correlation coefficient of the linear line and standard deviation of regression equation, standard deviation of slope, confidence limits of concentrations are presented in Table 2.

Limit of detection and limit of quantification were 1.7×10^{-6} M ($S/N = 3.3$) and 5.3×10^{-6} M ($S/N = 10$), respectively. These values are close to the results of a study which was achieved by capillary electrophoresis [8]. The performance of the method seems very reliable and it leads us to investigate the effect of intermediate precision.

3.4. Investigation of intermediate precision

A synthetic matrix was prepared consisting of corn starch and magnesium stearate as about 98 and 2%, respectively. It was finely mixed and 50-mg matrix was weighed to eighteen tubes which were divided into three groups.

A stock solution of KET was prepared and was added to the tubes of each groups corresponding to 50, 100 and 150% of a tablet amount. Similar procedures were performed without any matrix material which acted as a blank. The tubes were vigorously shaken were centrifuged at $5000 \times g$ supernatant was injected into the system. The results were tabulated in Table 3 which indicated no interference from the tablet excipients.

Table 1
The intra-day and inter-day precision test of KET using FIA detected at 260 nm, with the flow-rate of 0.6 ml min^{-1}

	Intra-day precision						Inter-day precision pooled ($n = 24$)	
	First day ($n = 8$)		Second day ($n = 8$)		Third day ($n = 8$)			
	Area	Height	Area	Height	Area	Height	Area	Height
Mean	934 713	133 883	938 676	132 077	933 837	134 694	935 742	133 551
SD	18 834	1126	20 352	1603	29 635	1426	22 493	1743
RSD	2.02	0.84	2.17	1.21	3.17	1.06	2.40	1.30

Table 2
Results of inter-day and intra-day calibration studies for linearity and accuracy of the method in the concentration range of 5.5×10^{-6} and 2.8×10^{-5} M

	Inter-day						Intra-day	
	First day ($n = 5$)		Second day ($n = 5$)		Third day ($n = 5$)		Whole days ($n = 15$)	
	Area	Height	Area	Height	Area	Height	Area	Height
Slope, a	1.7×10^{10}	2.4×10^{9}	1.7×10^{10}	2.4×10^{9}	1.7×10^{10}	2.4×10^{9}	1.7×10^{10}	2.4×10^{9}
Intercept, b	1×10^{4}	7.8×10^{2}	3.1×10^{3}	1.2×10^{3}	3.4×10^{3}	5.7×10^{2}	6.3×10^{3}	8.3×10^{2}
Correlation coefficient, r	0.9994	0.9999	0.9999	0.9999	0.9999	0.9999	0.9997	0.9999
SD of regression equation, $\pm Sr$	9.1×10^{3}	5.1×10^{2}	1.1×10^{4}	1.3×10^{2}	3.6×10^{3}	4.7×10^{2}	1.6×10^{4}	1.1×10^{3}
SD of the slope, Se	5.1×10^{8}	2.9×10^{7}	6.1×10^{8}	7.2×10^{6}	2.0×10^{8}	2.7×10^{7}	5.2×10^{8}	3.7×10^{7}
CL ($P = 0.05$)	$\pm 4.9 \times 10^{8}$	$\pm 2.7 \times 10^{7}$	$\pm 2.7 \times 10^{8}$	$\pm 6.8 \times 10^{6}$	$\pm 1.9 \times 10^{8}$	$\pm 2.5 \times 10^{7}$	$\pm 2.3 \times 10^{8}$	$\pm 1.7 \times 10^{7}$

Table 3
Intermediate precision of KET corresponding to 50, 100 and 150% of a tablet

	50% ($n = 6$)		100% ($n = 6$)		150% ($n = 6$)	
	Area	Height	Area	Height	Area	Height
Mean	98.33	98.86	98.94	97.92	97.92	99.98
SD	2.00	1.51	1.98	1.30	1.54	0.76
RSD	2.04	1.53	1.99	1.32	1.57	0.76
CL (0.05)	1.76	1.32	1.73	1.13	1.35	0.66

The RSD is within the range and this proves the validity of the proposed method.

Table 4
The statistical evaluations of the assay for KET tablets

	Peak area	Peak height
Mean	102.75	98.42
SD	1.51	1.81
RSD	1.47	1.84
CL	1.21	1.45

3.5. Application of the method to the pharmaceutical tablets

The determination of KET in pharmaceutical tablet (Profenid®, containing 200 mg KET) was carried out by the method described in this study. Tablets were processed as described in the experimental section. Six injections were made and the results are shown in Table 4 which indicate a satisfactory results for the proposed method.

Although no monograph in the USP XXIV [10] for KET tablet, however, the allowable range for other non-steroidal anti-inflammatory drugs of the profen class are mostly 90 and 110%. Therefore, the results found experimentally in this proposed method is in accordance with the official requirements.

In summary, the method proposed is simple, accurate, precise, rapid and it can be applied for the routine analysis of KET in the pharmaceutical formulation.

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