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Quantitative determination of ketoprofen in gels and ampules by using flow-injection UV spectrophotometry and HPLC

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Abstract

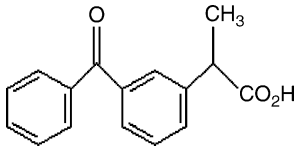
A flow-injection UV spectrophotometric method for the determination of ketoprofen in gels and ampules was developed. Quantitative determination of ketoprofen was realized by using distilled water as a carrier for gels and citrate buffer, pH 6.5, for ampules at 261 nm. No spectrophotometric interferences from additives of gels, carboxypolymethylene and triethanolamine, were observed. There were also no spectrophotometric interferences resulting from additives of ampules named as benzyl alcohol and arginine. The detection limits were 0.436 and 0.303 μg/ml for gels and ampules, respectively. Throughout the study, the flow rate, loop volume and the number of injection per hour were 13.8 ml min^{-1}, 193 μl and 85, respectively. Analytical signal of the ketoprofen was linear in the concentration range of 7.5–75 μg/ml. Quantitative results of ketoprofen in gels, 25.25 ± 0.27 (mean ± S.D.), and in ampules, 99.42 ± 0.44 were in good agreement with the labeled quantities (25 mg/1 g gel, 100 mg/2 ml ampule). The recoveries were in the range of 98.65–100.63 and 99.1–101.5% for gels and ampules, respectively.

Results obtained were in accordance with those obtained by HPLC. It was seen that the proposed method was fast, accurate, precise and suitable for automation as an analytical method.

Keywords: Ketoprofen; Flow-injection analysis; UV–vis spectrophotometry; Gels; Ampules

1. Introduction

Ketoprofen [2-(3-benzoylphenyl) propionic acid] is a nonsteroidal anti-inflammatory and analgesic agent.



Several methods have been described for ketoprofen determination in pharmaceutical formulations including UV spectrophotometry [1,2], chromatography [3–7], colorimetry [8,9]. In addition, there are some electrochemical [5,10], NMR [11] and FT-IR [12] methods used for the quantitative determination of ketoprofen. A different study has been conducted by Blanco et al. in that they eliminated the interference problem caused by paraben by using a derivative UV spectrophotometric method and performed the quantitative determination of ketoprofen and paraben [13]. Bonazzi et al. used SPE + UV spectrophotometric method in order to solve the interference in gels resulted from the preservatives [14]. In another study conducted by Donato et al., ketoprofen in ampules was determined using capillary chromatography [15]. Nowadays, flow-injection analysis has been extensively applied in routine analysis of pharmaceuticals [16–18].

This paper describes a flow-injection (FI) UV spectrophotometric method for the determination of ketoprofen in gels and ampules. Ketoprofen gels contain carboxymethylene, triethanolamine, ethyl alcohol and lavand oil. On the other hand, ketoprofen ampules are composed of arginine, benzyl alcohol and sodium citrate monohydrate as a buffer solution. Preservative activity is pH specific and benzyl alcohol is effective

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only in the pH range of 4–7 [19]. Since the ampule content contains citrate buffer, pH 6.5, and benzyl alcohol's stability is high at that pH value, citrate buffer was used as carrier solution.

Because flow-injection analysis is widely used in routine analysis of the pharmaceuticals and also there was no study using FI-UV spectrophotometric method for the determination of ketoprofen in gels and ampules in the literature, it was thought that the proposed method would be a useful technique for the determination of ketoprofen and the other pharmaceuticals having the similar composition. Furthermore, FI-UV spectrophotometric method was simple, rapid, sensitive and automated.

Quantitative determination of ketoprofen in gels and ampules was also performed using a new high-performance liquid chromatographic method as a reference method and results obtained using HPLC method were compared to those obtained by FI-UV spectrophotometric method.

2. Experimental

2.1. FI-UV spectrophotometric method

2.1.1. Apparatus

A Shimadzu UV-160A double-beam UV–vis spectrophotometer with data processing capacity was used. Spectral band width was 2 nm, and scan speed (slow mode) was set to 480 nm/min. And also, response time was 0.02 s. Proposed flow-injection methods were achieved by using a flow cell which was located in to the UV–vis spectrophotometer and a peristaltic pump helped maintaining the carrier flow. The flow-injection measurements were done using 10 mm quartz cells with 300 μl internal volume at 200–400 nm range and an ALITEA VS-3 10RI four channel peristaltic pump was used with Tygon, PharMed pink/white tubing throughout the study. Flow rate, loop volume and number of measurements per hour were 13.8 ml min^{-1}, 193 μl and 85, respectively. The pH measurements were performed by using a combined pH electrode with an Orion model 720 A. And also Juan MR 18.22 type centrifuge and a sonicator were used.

2.1.2. Reagents and solutions

Ketoprofen and pharmaceutical formulations containing ketoprofen (Profenid gel and Profenid ampule) were obtained from Eczacıbaşı Company in Turkey. Additives in gels and ampules were purchased from Sigma. All other reagents were analytical grade. The citrate buffer solution used as a solvent and carrier in ampules was prepared using 0.1 M sodium citrate monohydrate. Distilled water was used as a carrier in the determination of ketoprofen in gels. By dissolving appropriate amounts of ketoprofen in methanol, standard solutions of ketoprofen in the range of 7.5–75 μg/ml were obtained. Five hundred micrograms per milliliter carboxypolymethylene and 125 μg/ml triethanolamine solutions were also prepared in methanol. For the analysis of ketoprofen in ampules, standard solutions in the range of 7.5–75 μg/ml were obtained using citrate buffer solution, pH 6.5. Five hundred micrograms per milliliter benzyl alcohol and 1800 μg/ml arginine solutions were prepared with the citrate buffer solution.

2.1.3. Interference studies

2.1.3.1. Gels. Solutions of pure ketoprofen, gel and synthetic mixture of gel containing 10 μg/ml ketoprofen were prepared. In the preparation of synthetic mixture of the gel, 0.3 g carboxypolymethylene was weighed and 30 ml distilled water was added and then this mixture was incubated for one night at room temperature. After that, 20 ml (1.0 μg/ml) triethanolamine and 7.5 mg ketoprofen in 50 ml ethanol were added to the mixture. From this mixture, a solution containing 10.0 μg/ml ketoprofen was prepared. For the three solutions mentioned above, UV spectra were obtained in the 200–400 nm wavelength range.

2.1.3.2. Ampules. For the purpose of interference studies in ampule, again, three solutions were obtained from pure ketoprofen, ampule solution and synthetic ampule solution to investigate whether there was an interference effect or not coming from the additives. Apart from the gels, solutions were prepared in citrate buffer solution, pH 6.5. Synthetic ampule solution included 10 μg/ml ketoprofen, 5.0 μg/ml benzyl alcohol and 7.5 μg/ml arginine. By using UV spectrophotometer, absorption spectra of the three solutions containing 10 μg/ml ketoprofen were obtained in the wavelength range of 200–400 nm.

2.1.4. Optimization of FI conditions

Operating conditions, including loop volume, flow rate, number of injections per hour and type of tubing was carefully examined and optimum working conditions were determined. For this purpose, 56, 120, 138, 193 and 250 μl loop volumes were assayed. In order to optimize flow rate, 7.5, 9.38, 10.60, 12.58 and 13.8 ml min^{-1} flow rates were tested. While doing those tests in the optimization studies, peak shapes and absorbance values were taken in to consideration. The values providing high absorbance signals and well-shaped peaks were chosen as the working conditions. In addition, PharmaMed green–green, blue–blue and pink–white coded color tubings were tested.

2.1.5. Analysis of gels

Two-hundred milligrams of gel was accurately weighed, dissolved in 25 ml methanol and sonicated in an ultrasonic sonicator for 10 min and diluted to 50.0 ml with methanol. Twenty millilitres of this solution was then diluted to 100.0 ml with distilled water. The absorbance value of the solution at 261 nm in FIA system was obtained using distilled water as carrier solution. The ketoprofen content of gel was calculated by referring to a calibration curve obtained by using standard solutions of ketoprofen ranging from 7.5 to 75 μg/ml.

2.1.6. Analysis of ampules

0.5 ml ampule solution was diluted up to 50.0 ml with pH 6.5 citrate buffer. One milliliter of the solution was then diluted to 25.0 ml. The absorbance value of the solution at 261 nm in FIA system was obtained using citrate buffer solution as carrier. The ketoprofen content of ampule was calculated by referring to a calibration curve obtained by using standard solutions of ketoprofen ranging from 7.5 to 75 μg/ml.

2.2. HPLC

2.2.1. Apparatus

The HPLC system consisted of model HP series 1050 solvent delivery system with a UV–vis detector set to 261 nm. A HP ODS Hypersil column (10 cm × 3.9 mm. i.d., 5 μm particle size) and a HP 3396 series II integrator was used. Mobile phase filtration was performed with Erich Wiegand GmbH type N 022 AN 18 vacuum pump with All tech 47 mm, 0.45 μm filter paper. Typical operating conditions included flow rate, 1.2 ml min^{-1}; operating temperature, room temperature; injection volume, 20 μl; pressure, 80 bar.

2.2.2. Reagents and solutions

The mobile phase used in HPLC was phosphate buffer (pH 2.2, 0.01 M): acetonitril, 60:40, v/v. After mixing, the mobile phase was filtered through membrane filter and degassed with ultrasonic bath for 45 min. In order to prepare ketoprofen stock solution, 10.0 mg ketoprofen was accurately weighed, dissolved and diluted to 100.0 ml with methanol. Ten millilitres of the solution was then diluted to 100.0 ml with mobile phase. Standard solutions ranging from 0.4 to 1.2 μg/ml were prepared with the mobile phase. All solutions were prepared with bidistilled water. Acetonitrile was HPLC grade, Riedel-de Haen.

2.2.3. Analysis of gels

Twenty milligrams of gel was accurately weighed, dissolved in 25 ml mobile phase and sonicated in an ultrasonic sonicator for 10 min and diluted to 50.0 ml with mobile phase. One millilitre of this solution was then diluted to 10.0 ml with mobile phase. In order to determine ketoprofen content of gel, ketoprofen standard solutions were injected and calibration curve was obtained as peak area versus concentration. Twenty microliters of gel solution was injected and wavelength was set to 261 nm. By using calibration curve, quantitative determination of ketoprofen in gel was performed.

2.2.4. Analysis of ampules

One millilitre of ampule solution was diluted up to 50.0 ml with mobile phase and by taking 0.5 ml from this solution, it was diluted to 50.0 ml. One milliliter of the final solution was then adjusted to 10.0 ml. In order to determine ketoprofen content of ampul, ketoprofen standard solutions were injected and calibration curve was obtained as peak area versus concentration. Twenty microliters of ampule solution was injected and detection was conducted at 261 nm. By using calibration curve, quantitative determination of ketoprofen in ampule was performed.

3. Results and discussion

In this study, quantitative determination of ketoprofen in both pharmaceutical formulations, gels and ampules, was performed using a FI-UV spectrophotometric method and high-performance liquid chromatographic (HPLC) method.

3.1. Interference studies

Before conducting the quantitative determination of ketoprofen in both pharmaceutical forms, we studied the possibility of interference which would come from the additives. Additives present in gels are carboxypolymethylene and triethanolamine and they have absorbances at the UV region of the spectrum because of their functional groups. In order to determine whether there was an interference effect coming from these additives or not, we firstly obtained the UV spectra of ketoprofen standard solution, gel solution and the synthetic solution of the gel in the wavelength range of 200–400 nm, respectively. All the solutions were prepared in a way that concentration of the ketoprofen in the final solution was 10 μg/ml. When these three spectra were compared, it was seen that there was no difference between them and there didn't exist any interference effect caused by the additives.

Benzyl alcohol and arginine are the two additives present in ampules of ketoprofen. For the purpose of determining if there was any interference effect coming from these two additives, same procedures used in gels were applied and after examining the three spectra, it was concluded that there was not any interference effect caused by the additives present in the ampule in the determination of ketoprofen.

3.2. Optimization studies

In order to perform FI-UV spectrophotometric determination of ketoprofen in gels and ampules, flow-injection parameters were optimized. As it can be seen from Table 1, optimum conditions for the loop volume, flow rate and type of tubing and wavelength were determined. The effect of sample volume was investigated by inserting loops of dif-

Table 1
Optimization of the flow-injection parameters

Parameters	Tested conditions	Optimum condition
Loop volume (μl)	56, 120, 138, 193, 250	193
Flow rate (ml min^{-1})	7.50, 9.38, 10.60, 12.58, 13.80	13.8
Tubing (type)	PharMed green–green, blue–blue, pink–white	PharMed Tygon, pink–white
Wavelength (nm)	200–400	261

ferent volumes between 56 and 250 μl. Higher sensitivity was obtained by using a larger volume of sample solution. Because small loop volumes caused low absorbance values, wide peaks and more time was required for each determination, by taking in to consideration both sensitivity and analysis speed, 193 μl was choosen as the optimum loop volume. For the optimization of flow-rate, five values were studied. Among them, 13.8 ml min^{-1} gave the best results because the other tested flow rates caused low absorbance values and wide peaks. In addition, lower the flow-rate, the lower the sampling frequency. Lastly, for the selection of best tubing, a variety of tubing was tested and PharmMed Tygon pink–white was selected since it provided optimum flow rate for the carrier solution.

3.3. Ketoprofen determination studies

Since there was no spectrophotometric interference resulting from additives of both gels and ampules, it was decided to conduct quantitative determination of ketoprofen using FI-UV spectrophotometric method. At 261 nm, a linear relationship was observed between the ketoprofen concentration and absorbance values. Fig. 1a and b show signals in the determination of ketoprofen in gels and ampules, respectively. Signals were obtained by injecting 193 μl of the ketoprofen standard solutions and the calibration curve was reasonably linear in the concentration range of 7.5–75 μg/ml.

The linearity of the calibration graph and the adherence of the system to Beer's Law were validated by the high values of the correlation coefficient of the regression equation, 0.9995 for gels and 0.9999 for ampules, respectively. In addition, the

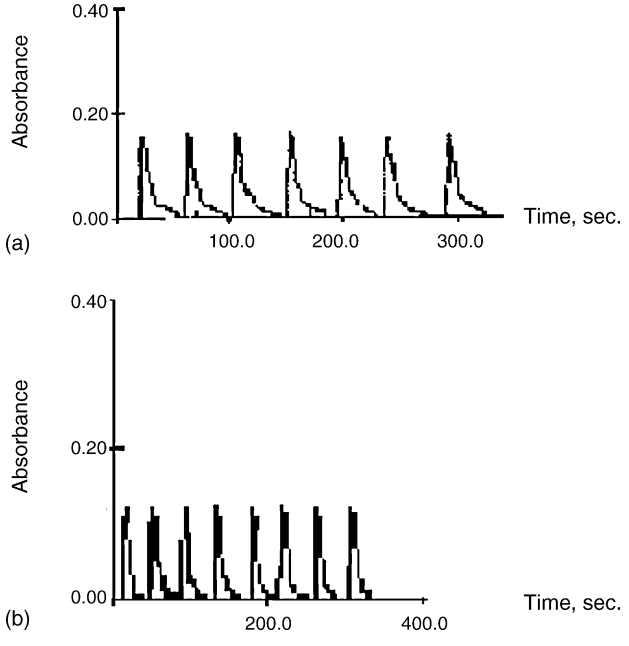

Fig. 1. FI-UV spectrophotometric spectrum of gel solution containing 20 μg/ml ketoprofen (a) and FI-UV spectrophotometric spectrum of ampule solution containing 20 μg/ml ketoprofen (b).

Table 2
Analytical parameters for the FI and HPLC methods

	HPLC	FI
Gels		
Linearity range	0.4–1.2	7.5–75
Correlation coefficient	0.9997	0.9995
Equation of calibration curve[a]		
Slope	196111	7.56×10^{-3}
Intercept	7889	6.80×10^{-3}
LOD	0.05	0.44
LOQ	0.16	1.32
R.S.D. (%)[b]	1.27	1.07
Sampling frequency (h^{-1})	15	85
Ampules		
Linearity range	0.4–1.2	7.5–75
Correlation coefficient	0.9997	0.9999
Equation of calibration curve[a]		
Slope	196111	6.27×10^{-3}
Intercept	7889	1.27×10^{-3}
LOD	0.05	0.303
LOQ	0.16	0.92
R.S.D. (%)[b]	1.23	0.44
Sampling frequency (h^{-1})	15	85

[a] $y = mx + n$, where x is the concentration in μg/ml.
[b] Calculated from five determinations of readings from gel and ampule. LOD = 3.3 (S.D./slope); LOD = 10 (S.D./slope).

experimental intercept was not significantly different from the theoretical zero value. High correlation coefficient values and low relative standard deviations, 1.07% for gels and 0.44% for the ampules, proved the precision of the proposed method, Table 2. Standard solution of ketoprofen which has lowest concentration, 7.5 μg/ml, was measured five times to calculate the limit of detection as well as limit of quantification for both gels and ampules. And, calculated LOD values for gels and ampules were 0.436 and 0.303 μg/ml, respectively.

Quantitative determination of ketoprofen in gels and ampules were performed using FI-UV spectrophotometric method and compared to those obtained by reference method, HPLC. Results obtained were in good agreement with the labelled amounts of ketoprofen and values found using HPLC, Table 5. In addition, as far as % R.S.D. values of both methods concerned, the proposed method had better precision. This result was more apparent especially in the determination of ketoprofen in ampules, 0.44 for FI-UV spectrophotometric method and 1.23 for HPLC method, Table 2.

3.4. Recovery studies

In order to test the accuracy of the proposed methods, recovery studies and reference method were used. Recovery studies on the proposed methods were performed by spiking the sample of the gels and ampules with appropriate amount of the stock solution of the ketoprofen. As can be seen from Table 3, high recovery values, 99.18% for gels and 99.58% for ampules, showed the accuracy of the proposed method.

Table 3
Recovery analyses of ketoprofen

	Amount labeled	Added (mg)	Recovery (mg)[a]	Recovery (%)	S.D.
FIA					
Gels	25 mg/g	12.5	12.40	99.18	0.86
Ampules	100 mg/2 ml	50.00	49.79	99.58	0.71
HPLC					
Gels	25 mg/g	12.5	12.55	100.4	1.44
Ampules	100 mg/2 ml	50.00	50.05	100.1	1.14

[a] This is the mean of three experiments.

Table 4
Intra-day and inter-day precision of ketoprofen standards using FI

Concentration of ketoprofen (μg/ml)	Intra-day measured concentration (μg/ml)[a]		Inter-day measured concentration (μg/ml)[b]	
	Mean	R.S.D. (%)	Mean	R.S.D. (%)
30 (in distilled water)	29.52	0.0	29.56	0.25
30 (in citrate buffer)	30.18	1.04	32.52	0.28

[a] Mean concentration represents five different ketoprofen standards for each concentration.
[b] Inter-day mean values were determined from five different runs over 1-week period.

Furthermore, recovery values obtained using the proposed methods were in good agreement with the reference method. Table 4 represents the results obtained in intra-day and inter-day variability studies of the ketoprofen in gels and ampules. The proposed methods were examined through 193 μl injections of 30 μg/ml ketoprofen solutions. The observed relative standard deviations were 0.0% in intra-day measurement and 0.25% in inter-day measurements for the gels. For the ampules, these values were 1.04 and 0.28%, respectively. These results show the accuracy and repeatability of the proposed methods, which were tested within day and between days.

3.5. HPLC as a reference method

Quantitative determination of ketoprofen in gels and ampules was also conducted using a HPLC method. Standard ketoprofen solutions were eluted, forming well-shaped, symmetrical single peak and well separated from the solvent front. The HPLC method used was a new modified method. Recovery values obtained using HPLC were 100.4% for gels and 100.1% for ampules. It was seen that the results obtained with HPLC method were in good agreement with those obtained by FI-UV spectrophotometric method, Table 3. But, standard deviations in this method were higher than the proposed method.

Results obtained by the proposed methods and HPLC were compared by Student's *t*-test and Fisher test. When the precisions of the both methods were compared, there was no significant difference, at 95% confidence level, between the two methods for both gels and ampules, Table 5. In addition, as far as student's *t*-test results were concerned, there did not also exist any significant difference between two methods with respect to mean values, Table 5. This conclusion showed also accuracy of the proposed flow-injection method.

Table 5
Statistical comparison of the two methods

	FIA method	HPLC method
Amount labeled (25 mg/g gel)		
Amount found (mg/g gel), average values ($n = 5$)	25.25	25.14
$t_{calculated} = 0.59$	$t_{theoretical} = 2.31$ ($p = 0.05$)	
$f_{calculated} = 1.40$	$f_{theoretical} = 6.39$ ($p = 0.05$)	
Amount labeled (100 mg/2 ml ampule)		
Amount found (mg/2 ml ampule), average values ($n = 5$)	99.42	99.30
$t_{calculated} = 0.20$	$t_{theoretical} = 2.31$ ($p = 0.05$)	
$f_{calculated} = 6.25$	$f_{theoretical} = 6.39$ ($p = 0.05$)	

As a result, the proposed flow-injection UV spectrophotometric method was simple, automated and suitable for the routine analysis of the ketoprofen in gels and ampules.

4. Conclusion

It can be concluded that FI-UV spectrophotometric method has the advantage of being simpler, more rapid, suitable for automation. The proposed method had a relatively high sampling rate, 85 h^{-1}, compared to conventional HPLC method, 15 h^{-1}. In addition, recovery studies performed by FI-UV spectrophotometric method show the accuracy of the proposed method, 99.18%, for gels and 99.58%, for ampules. Furthermore, as far as detection limits are concerned, 0.396 μg/ml for gels and 0.276 μg/ml for ampules, the proposed method is sensitive to the low amounts of ketoprofen.

As a result, the proposed FI-UV spectrophotometric method represents a good analytical alternative for the determination of ketoprofen in gels and ampules.

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