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LC method development for ibuprophen and validation in different pharmaceuticals

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Abstract

An isocratic LC method for the simultaneous determination of ibuprophen (IBU) and its degradation product (4-isobutylacetophenone) has been developed and validated for different pharmaceuticals (sachets, tablets and gels). The chromatographic separation was achieved with phosphoric acid solution (pH 3.2)-acetonitrile (50:50, v/v) as mobile phase, a Hypersil C_{18} column and UV detection at 254 nm. In all cases, sample preparation was required before HPLC analysis.

Keywords: LC; Ibuprophen; Pharmaceuticals; Validation

1. Introduction

Ibuprophen (IBU) [2-(4-isobutylphenyl) propionic acid] is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic and platelet antiagregant properties, which is better tolerated than aspirin, indomethacin and pirazolonic derivatives. In addition, it is well absorbed from the gastrointestinal tract following oral or rectal administration. For these reasons, it is recommended for the symptomatic relieve of certain rheumatic diseases and to relieve moderated pain, acute arthritis, nonrheumatic inflammations, fever and dysmenorrehea [1,2].

Several HPLC methods for IBU determination have been described in the literature. These methods have been applied to the determination of IBU in pharmaceutical preparations using different internal column diameters [3], when associated with other compounds, such as chlorzoxazone [4] or paracetamol and chlorzoxazone [5], and in biological samples, such as human plasma [6–8] and human urine [9]. Other studies for IBU determination have also been carried out in biological samples, such as serum by capillary electrophoresis [10] and in human urine samples by GC–MS [11] and HPTLC [12].

The US Pharmacopoeia describes two different RP-HPLC methods, one for impurities in raw materials using UV detection at 214 nm, and another one for IBU determination in pharmaceu-

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ticals and in raw materials using UV detection at 254 nm. With regard to the related substances test, the area of an individual secondary chromatographic peak which appears in the test sample should not exceed 0.3% of the IBU peak area, and the total area of all secondary peaks, 1% IBU peak area (the compounds are not specified) [13]. A similar HPLC method has been described for IBU determination in the British Pharmacopoeia (BP) in pharmaceuticals and raw materials [14]. BP specifies five substances as possible impurities [one of them 4-isobutyl acetophenone (IKP)] and indicates the importance of control of 2-(4-butylphenyl) propionic acid (BPA) in raw materials, whose content should not exceed 0.3% of IBU peak area. In addition, if secondary peaks appear, their individual area must not exceed 0.3% and the corresponding total must be lower than 0.7% of the IBU peak area. The control of IKP is indicated only in oral suspensions. In the Spanish Royal Pharmacopoeia, a similar control is indicated [15].

It is well known that IKP causes adverse effects in the central nervous system and presents high dermal absorption. For these reasons, its control is necessary, especially for gels (a recent pharmaceutical presentation).

However, it is worth noting the lack of official methods for assays of active ingredients in pharmaceutical preparations in refuted pharmacopoeias, moreover, when it is currently possible to find a plethora of methods based on RP-HPLC for the determination of these compounds. As an example, Ghosh [16] has described 1.300 HPLC methods for hundreds of active ingredients. However, only a few of the proposed methods have been adequately validated [17–19].

In this paper, a simple, sensitive, precise and rapid HPLC method for the simultaneous determination of ibuprophen and its main degradation product IKP is reported. It is shown that the developed method is adequate for the determination of these compounds in different pharmaceutical preparations, such as sachets, tablets and gels. Finally, the method is validated for each pharmaceutical.

2. Experimental

2.1. Chemicals and reagents

IBU and valerophenone (VP) were from Sigma (St. Louis, MO). 4-(isobutylacetophenone) (IKP), 2-(4-*n*-butylphenyl) propionic acid (BPA) and 2-(3-isobutylphenyl) propionic acid (IBPA) were from Albemarle Corporation (Baton Rouge, LA). HPLC-grade acetonitrile were from Promochem (Wesel, Germany). A water–acetonitrile (50:50, v/v) mixture was used to prepare standard solutions of IBU, IKP, BPA, IBPA and VP. Phosphoric acid was of analytical-reagent grade from Merck (Darmstadt, Germany). Water was purified with a Milli-Q system (Millipore, Molsheim, France). Millipore 0.45 μm nylon filters (Bedford, MA) were also used. Other chemicals used were of analytical reagent grade.

2.2. Apparatus

The chromatographic system consisted of the following components, all of them from LDC Analytical (Riviera Beach, FL): a Constametric 4100 solvent delivery system, a spectromonitor 5000 photodiode-array detector, covering the range 190–360 nm and interfaced to a computer for data acquisition, recorder model CI 4100 data module. A Rheodyne 20 μl loop injector (Cotati, CA), a Jones-Chromatography block heated series 7960 (Seagate Technology, Scotts Valley, CA) for thermostating columns and a Hypersil ODS, bonded-silica, (150 × 3.0 mm I.D.) 5 μm column from Phenomenex (Torrance, CA) and a vortex mixer Mixo-Tub-30 from Crison (Barcelona, Spain) were used. For pH measurements, a Crison digital pH-meter with an Ingold 10-402-253 glass combined electrode was used. A ultrasonic bath from Selecta (Barcelona, Spain) was also used.

2.3. Mobile phase

The mobile phase consisted of a phosphoric acid solution (pH 3.2)-acetonitrile (50:50, v/v). HPLC-grade acetonitrile and water were previously filtered under a vacuum through 0.45 μm nylon filters and degassed using helium sparge.

2.4. Chromatographic analysis

Once the column had been conditioned with the mobile phase at 40°C (for reproducible measurements), the standard solutions containing IBU, IKP and IS and sample solutions were injected (20 μl). The flow-rate was 0.5 ml min^{-1} and UV-diode array (DAD) detection in the range 190–360 nm was employed. Identification of compounds and peak purity were performed by comparison between the UV spectra of the chromatographic peaks with those of compounds previously registered by injection of each one individually. Analyses were monitored at 254 nm.

2.5. Sample preparation

Standard solutions of IBU 10000 μg ml^{-1}, IKP 2000 μg ml^{-1} and VP (IS) 10 μg ml^{-1} were prepared as indicated in Section 2.1. VP solution (10 μg ml^{-1}) was used to prepare samples and more dilute standards.

Ten tablets (or the contents of ten sachets) were adequately pulverized to obtain a fine powder. A total of 300 mg (sachets) or 32 mg (tablets) from the homogeneous mixture, containing 20 mg IBU each, were adequately weighted and treated with IS solution (10 ml). After adequate agitation, the mixture was sonicated for 5 min to produce the complete dissolution of the interest compounds. The resulting solution was filtered through 0.45 μm nylon filters and an aliquot of 1 ml was diluted using IS solution up to a final volume of 5 ml.

Ten different gel samples of similar weight were adequately mixed. A total of 80 mg (4.0 mg IBU) from the homogeneous mixture was adequately weighted in a stoppered centrifuge test tube. A total of 8.0 ml of IS solution was added and the mixture shaken vigorously for 2 min and then sonicated for 5 min. The mixture was later centrifuged for 3 min at $3700 \times g$, and the supernatant solution was transferred to a volumetric flask (10 ml) and its volume was completed using IS solution.

All samples were filtered using nylon filters (0.45 μm) and 20 μl were injected directly on the chromatographic column. In all cases, the theoretical IBU concentration was 400 μg ml^{-1} (100% IBU). Placebo samples were prepared by weighting and mixing the excipients of each pharmaceutical, and were processed in a similar way to the pharmaceuticals.

3. Results and discussion

3.1. Chromatographic optimization

A mobile phase phosphoric acid solution (pH 3.2)-acetonitrile was used, in order to optimize the chromatographic separation of IBU and possible degradation products (DPs): [(4-isobutylacetophenone (IKP), 2-(4-*n*-butylphenyl) propionic acid (BPA), 2-(3-isobutylphenyl) propionic acid (IBPA)] and VP (IS), with adequate characteristics, such as resolution and short time analysis. Fig. 1 shows the structure and UV absorption spectrum of IBU and related compounds. Among the different contents (40–60%), 50% of acetonitrile was finally selected. In these conditions, a separation up to base line including all compounds above mentioned, was achieved. Fig. 2 shows the chromatograms obtained at 220 nm (A) and 254 nm (B) with this mobile phase using a standard mixture containing 10 μg ml^{-1} of IBU, DPs and VP. For further studies, UV detection at 254 nm was selected (see Fig. 1) as adequate for the simultaneous detection of IBU and IKP compounds, e.g. in pharmaceuticals. In this way, IKP can be detected at low concentrations in the presence of IBU at high concentrations.

3.2. Separation performances

The separation obtained from a standard sample containing IBU (400 μg ml^{-1}), IKP (0.2 μg ml^{-1}) and IS (10 μg ml^{-1}) is shown in Fig. 3(A). Estimates of the mean and RSD values ($n = 6$), using peak area ratios, are listed in Table 1. The RSD ($n = 6$) of the retention factors, k, for the compounds was lower than 1% for each one. As can be observed, the data obtained from these compounds are adequate to develop an analytical method [20].

3.3. Calibration graphs and detection limits

Standards containing mixtures of IBU and IKP were prepared at eight different concentrations using VP as IS in the range shown in Table 2. These solutions were analyzed with the optimized mobile phase, using a flow-rate of 0.5 ml min^{-1}, a Hypersil ODS column and UV-DAD detection at 254 nm. The calibration equation, $Y = A + Bx$ (μg ml^{-1}), was obtained for IBU and IKP by plotting peak area ratios of IBU/IS or IKP/IS (Y) versus the concentration (x). In Table 2, parameters A (intercept), B (slope) and r (regression coefficient) are given.

The detection limits (LDs) obtained for a signal-to-noise (S/N) ratio of 3 are also shown in Table 2.

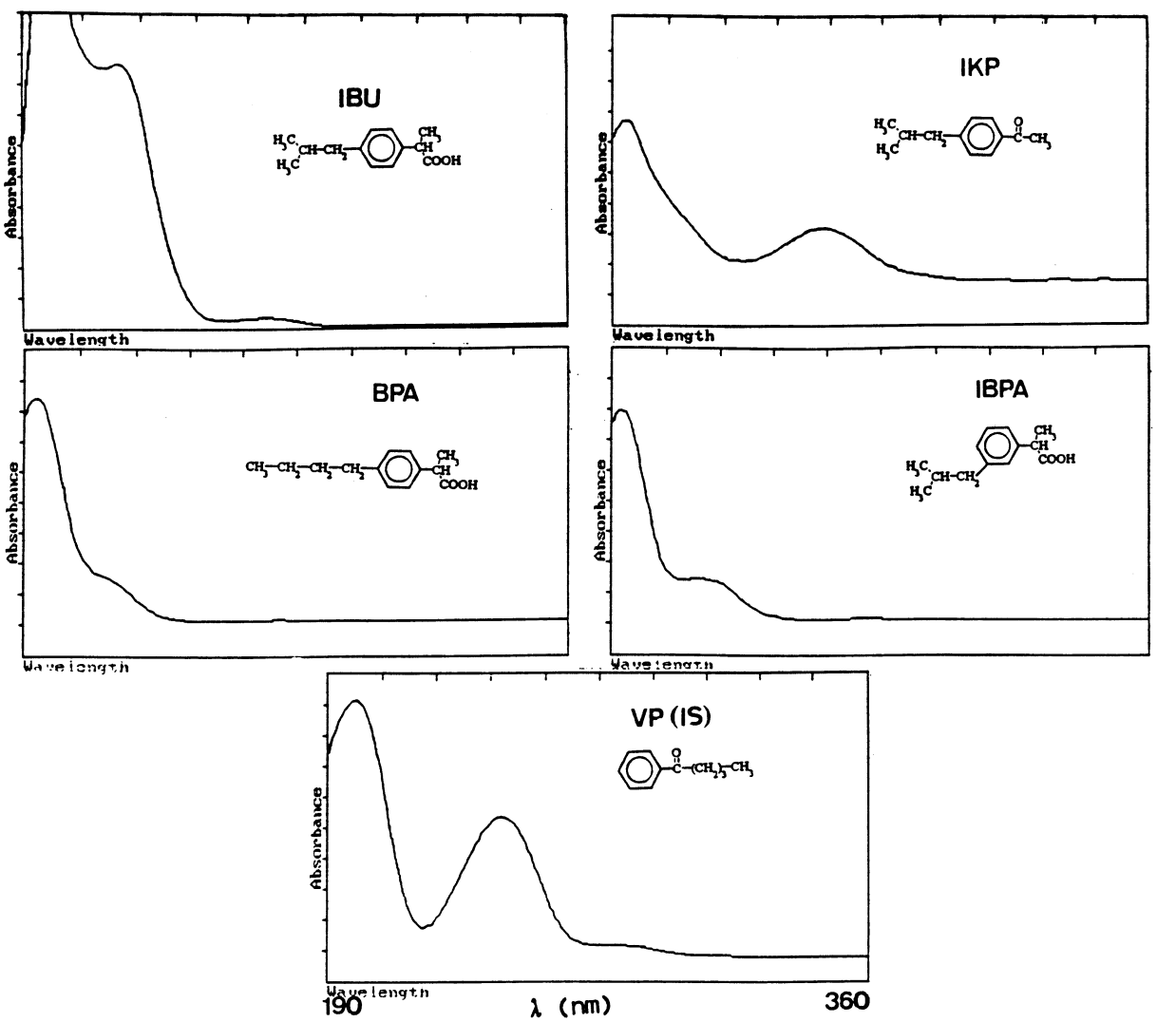

Fig. 1. Chemical structures and UV spectra (range 190–360 nm) obtained from IBU and related compounds: IKP (4-isobutylacetophenone); BPA (2-(4-*n*-butylphenyl) propionic acid; IBPA (2-(3-isobutylphenyl) propionic acid and valerophenone (VP) used as IS.

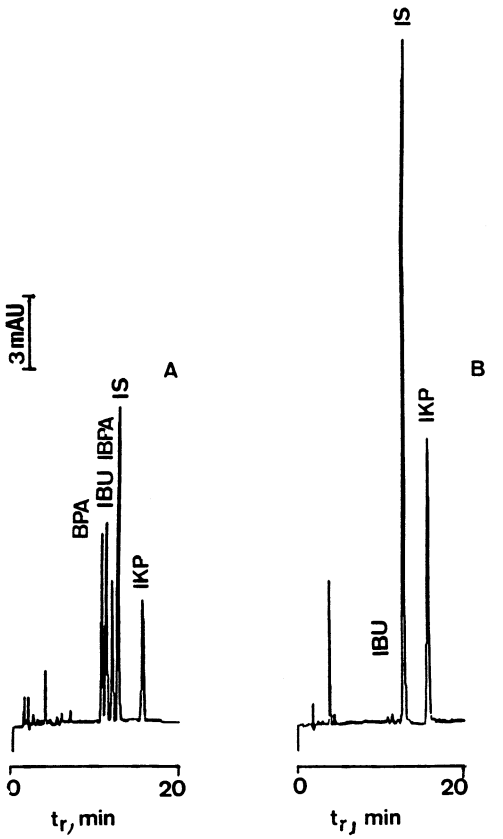

Fig. 2. Chromatograms obtained with UV detection at 220 nm (A) and 254 nm (B) from a standard mixture containing 10 μg ml^{-1} of IBU, IKP, BPA, IBPA and VP (IS). Chromatographic conditions: Hypersil C_{18} column and phosphoric acid solution (pH 3.2)-acetonitrile (50:50, v/v) as mobile phase.

4. Analysis of pharmaceuticals and validation method

4.1. Extraction of active ingredients

Adequate sample preparation was required prior to injection of pharmaceutical and placebo samples into HPLC. For IBU extraction from samples (gels or powders), a VP (IS) solution [(prepared with water–acetonitrile (50:50, v/v)] was added using the required volume and the mixture shaken vigorously and sonicated. The mixture was then centrifuged and the resulting solution removed from the test tube (as indicated in Section 2.5 for sample preparation) (14). As representative examples, Fig. 3 shows the chromatograms obtained with UV detection at 254 nm using a standard mixture of IBU and IKP (Fig. 3(A)), and a gel sample (Fig. 3(B)) after sample preparation. The recoveries ($n = 6$) obtained by

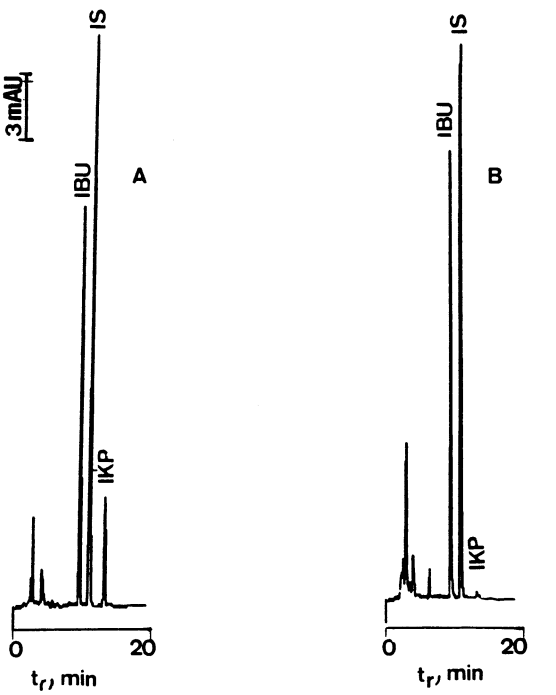

Fig. 3. Chromatograms obtained with UV detection at 220 nm: (A) from a standard solution containing IBU (400 μg ml^{-1}), IKP (0.2 μg ml^{-1}) and VP (IS) (0.2 μg ml^{-1}), and (B) from a processed gel sample. Chromatographic conditions as in Fig. 2.

Table 1
Performances of the HPLC separation obtained from Fig. 2(A) involving IBU, VP (IS) and IKP[a]

Parameter	IBU	VP (IS)	IKP
k	3.74	4.63	5.81
N	826	1600	6002
ASF	1.01	1.00	1.00
R_S	1.4		2.6
RSD (%)	0.79	—	3.60

[a] Conditions as in Fig. 2(A) where: k, is the retention factor; N, the number of theoretical plates of the column; ASF, the asymmetry factor of the peaks; R_S, the resolution between the peaks; α, the separation factor; and RSD, the relative standard deviation of peak areas.

Table 2
Linear regression equations (PAR = $A + Bx$) and detection limits (LDs) for IBU and IKP[a]

	Range (μg ml^{-1})	A	B	r	LDs (μg ml^{-1})
IBU	10–450	$-7 \cdot 10^{-4}$	$1.4 \cdot 10^{-3}$	0.9991	0.98
IKP	0.10–500	2.24	0.048	0.993	$1.9 \cdot 10^{-4}$

[a] PAR, peak area ratio of IBU or IKP to VP (IS) 4 μg ml^{-1}; x, μg ml^{-1} of IBU and IKP; r, correlation coefficient.

means of the calibration curves for IBU in tablets, sachets or gels were (100.2 ± 2.6), (100.4 ± 2.2) and (99.8 ± 1.1), respectively.

4.2. Linearity

A similar calibration to the one performed above was carried out in the concentration range of interest for the determination of IBU and IKP contained in the pharmaceutical preparations. The linearity test was performed using seven different amounts of IBU in the range of 80–120% around the theoretical values (400 μg ml^{-1}) and in the range 0.05–0.45% of IBU content for IKP. The correlation coefficients, r, found were 0.9995 and 0.9998 for IBU and IKP, respectively, which were higher than those obtained in Section 3.3. In that case, the concentration range used was wider.

4.3. Precision (repeatability and reproducibility)

The precision was examined by analyzing six different samples of each pharmaceutical preparation ($n = 6$) by only one operator, applying the proposed method from the calibration curves. The repeatability (within-run precision) was evaluated by only one analyst within 1 day, whereas reproducibility (between-run precision) was evaluated for 3 different days. The results obtained are in Table 3.

4.4. Accuracy

Placebo samples were spiked with different amounts of IBU at 90, 100 and 110% in triplicate for each one ($n = 9$) over the theoretical values and IKP at 0.1% over the IBU content. The mixtures obtained were processed according to sample preparation method (Section 2) and IBU and IKP were determined. The mean values of the percent recoveries obtained are shown in Table 4. As expected, the recovery values for IBU are consistent with those obtained above (Section 4.1).

4.5. Selectivity

Selectivity was assessed by a qualitative comparison between chromatograms obtained from samples and placebos. Possible interferences present in pharmaceuticals were not observed. In addition, the presence of other possible DPs can

Table 3
Within and between-run precision for pharmaceutical preparations containing IBU and IKP

Samples content: Mean (mg g^{-1}); RSD (%)		
Pharmaceutical	IBU	IKP
Within-run precision		
Sachets	121.6; 1.8	0.018; 6.1
Tablets	619.3; 0.89	0.028; 6.40
Gels	49.04; 1.47	Undetected
Between-run precision		
Sachets	125.3; 2.99	0.020; 11.3
Tablets	630.81; 1.61	0.029; 9.70
Gels	50.31; 3.21	Undetected

Table 4
Accuracy test for pharmaceutical preparations containing IBU and IKP

Pharmaceutical	IBU	IKP
Recoveries (%); RSD (%)		
Sachets	100.4; 1.8	100.1; 2.0
Tablets	100.4; 2.8	97.3; 1.8
Gels	100.6; 1.0	98.13; 2.2

Table 5
Robustness test for pharmaceuticals containing IBU and IKP carried out by three operators[a]

Sample content: Mean (mg g^{-1}); RSD (%)			
Operator	Pharmaceutical	IBU	IKP
1	Sachets	121.6; 1.8	0.018; 6.1
	Tablets	619.3; 0.89	0.028; 6.4
	Gels	49.04; 1.5	Undetected
2	Sachets	131.5; 2.9	0.020; 9.5
	Tablets	622.2; 2.1	0.028; 13
	Gels	49.12; 1.5	Undetected
3	Sachets	133.1; 5.8	0.018; 9.4
	Tablets	627.9; 2.1	0.028; 13
	Gels	49.84 3 6	Undetected
Mean	Sachets	128.7; 3.9	0.019; 8.8
	Tablets	623.1; 1.9	0.028; 11
	Gels	49.3; 3.1	Undetected

[a] Conditions for the operator 2 [mobile phase, phosphoric acid solution (pH 3.3)-acetonitrile (49%), flow-rate 0.6 ml min^{-1}, column temperature 38°C and UV detection, at 252 nm)] and operator 3 [mobile phase, phosphoric acid solution (pH 3.1)-acetonitrile (51%), flow-rate 0.4 ml min^{-1}, column temperature 42°C and UV detection, at 256 nm].

not cause interference in the IBU determination because the detection was at 254 nm (see absorption spectra from Fig. 2(B), Fig. 3(B)).

4.6. Robustness

In order to test the robustness of the method, six samples were analyzed by two operators (2 and 3) using standards prepared by themselves and under different chromatographic conditions than those used in the present method (operator 1). The working conditions for the operators and the results obtained are in Table 5.

5. Conclusions

The HPLC method described in this paper achieves the established pharmacopoeias requirements to be used as a routine method for the quality control and stability studies of pharmaceutical products. The validation study carried out over three different pharmaceutical presentations afforded acceptable quality parameters. In addition, the method could be applied to the analysis of IBU and related compounds with different purposes, such as testing raw materials or dissolution speed assays. Moreover, the proposed HPLC method has some advantages in comparison with the methods described in pharmacopoeias (USP, BP): it allows the simultaneous determination of IBU and IKP in different kind of samples (tablets, sachets and gels), the analysis time is shorter and the mobile phases used have better performances.

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