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# TLC Chromatographic–Densitometric Assay of Ibuprofen and Its Impurities

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## Abstract

**A simple and sensitive thin-layer chromatographic (TLC)–densitometric method for the quantitative estimation of S(+)-2-[4-isobutylphenyl]propionic acid (ibuprofen) and its impurities in pharmaceutical preparations has been developed. The chromatographic separation was carried out on silica gel 60 $F_{254}$ TLC plates using toluene–ethyl acetate–glacial acetic acid (17:13: 1, v/v/v) as the mobile phase. Detection was carried out densitometrically with a UV detector. The developed method has detection and quantitation limits ranging from 0.13 µg per spot to 0.72 µg per spot. For individual constituents the recovery ranged from 96.8% to 99.0%. In addition, the stability of ibuprofen solutions was investigated, including the effect of pH, temperature, and incubation time. The method is rapid, simple, and suitable for routine quality-control analysis of pharmaceuticals containing ibuprofen.**

## Introduction

S(+)-2-[4-isobutylphenyl]propionic acid (S(+)-ibuprofen) belongs to a class of nonsteroidal drugs inhibiting the activity of the enzyme cyclooxygenase and has strong analgesic and antipyretic action (1). It is used in the treatment of pain which coexists with inflammation. It is widely used in the treatment of pain from rheumatic illnesses, myalgias, post-traumatic neuralgias, migraine headaches, fevers, head colds, and the symptoms of influenza.

For studying the quality of medicines with ibuprofen, various pharmacopoeias recommend different analytical methods such as spectroscopy (IR and UV) and the thin-layer chromatography (TLC) on silica gel plates with anhydrous acetic acid–ethyl acetate–hexane (5:24:71, v/v/v) as mobile phase (2,3). For the estimation of drug purity, liquid chromatography (LC) is the recommended method.

Survey of the literature reveals various methods available for the determination of ibuprofen. The methods include high-performance liquid chromatography (HPLC) (4–8), gas chromatography (GC) (4,9), capillary isotachophoresis (10), spectrofluorometry (11), and potentiometric titration in non-aqueous media (12). A high-performance thin layer chromatography (HPTLC) method with *n*-hexane–ethyl acetate–anhydrous acetic acid as mobile phase, for quantitation of ibuprofen from human plasma was described (13). The application of tandem mass spectrometry to the analysis and identification of paracetamol, ibuprofen, and indomethacin following TLC with silica gel and diol-bonded silica gel HPTLC plates, in biological samples was reported (15). HPLC and TLC (supplemented with UV spectrometry) methods for simultaneous determination and separation of paracetamol, ibuprofen, and diclofenac from commercial formulations were presented. They were extracted, isolated, purified, and recrystallized, and they were characterized by melting point, $\lambda_{max}$ and IR (5). TLC with videodensitometry for separation and identification of fenbufen, ibuprofen, ketoprofen, diclofenac sodium, mefenamic acid and tiaprofenic acid on normal-phase and reversed-phase plates has been presented (15).

TLC is the most simple and basic analytical procedure and is used for the separation of widely applicable compounds. A TLC method has become useful as a technique due to its advantages of reliability in quantitation of analytes at micro or nanogram levels and its cost effectiveness. The major advantage of TLC is that several samples can be analyzed using a small quantity of mobile phase. This reduces the time and cost of analysis. TLC–densitometry also facilitates repeated detection of the components of the chromatogram with the same and different parameters. Moreover, the method enables simultaneous analysis of several compounds. TLC is comparable with other methods used for drug analysis, for example HPLC and GC.

Through continuing research of the efficiency of methods for the chromatographic determination of non-steroidal anti-inflammatory drugs and the significance of the sensitivity and reliability of TLC in the analysis of pharmaceutical preparations (16–18), an attempt was made to develop a novel, simple, rapid, and validated TLC–denitometry method, which considerably improved the efficiency for ibuprofen estimation in drugs. The present study established the conditions for identification and quantitation of ibuprofen in presence of its impurities, 2-[4-isobutyrylphenyl]propionic acid and 2-[4-isobutylphenyl]-propionamide (2,3). In addition, the effect of pH, temperature and incubation time on ibuprofen stability was investigated.

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## Experimental

### Apparatus

All designed experiments were carried out with a densitometer TLC Scanner 3 with Cats 4 software, (Camag, Muttenz, Switzerland). Solutions were applied by sample applicator Linomat V, (Camag). Silica gel aluminium TLC $F_{254}$ plates, art. No 1.05554 were obtained from E. Merck, Darmstadt, Germany. Chromatograms were developed in a TLC chamber of 18 × 9 × 18 cm in size (Sigma-Aldrich, St. Louis, MO). $^1$H NMR analysis were carried out on a spectrometer NMR Mercury VX 300MHz, (Varian, Palo Alto, CA).

### Reagents and chemicals

*Standard substances*

S(+)-2-[4-isobutylphenyl]propionic acid (S(+)-ibuprofen; Fluka), 2-[4-isobutyrylphenyl]propionic acid (impurity B) (STADA) and 2-[4-isobutylphenyl]propionamide (impurity A) (STADA) were used.

*Reagents*

Methanol, (Merck), toluene, ethyl acetate, (POCH, Gliwice, Poland), and glacial acetic acid (Odcz. Sp.z o.o., Poland) were used. All the reagents were of analytical grade.

*Samples*

The following medicines were analyzed: Ibuprom, tablets containing 200 mg of ibuprofen (US Pharmacia Int., Rockville, MD); Ibuprofen, tablets containing 200 mg of ibuprofen (Polfa Pabianice, Pabianice, Poland); Nurofen, tablets containing 200 mg of ibuprofen (Boots Healthcare Int., Nottingham, UK); Nurofen, granulate containing 200 mg of ibuprofen per 2.1 g of granulate (Boots Healthcare Int.); Ibufen, suspension containing 100 mg of ibuprofen per 5 mL of suspension (Terpol, Warszwa, Poland).

### Standard and sample solutions

*Standard solutions*

Solutions of concentration 0.1 mg/mL of ibuprofen, impurity A, and impurity B in methanol were prepared.

*Sample solutions*

*Tablets.* Ten tablets were finely ground in a mortar; an amount corresponding to 10 mg of ibuprofen was weighed and then 10.0 mL of methanol was added and shaken for 30 min; the solution was filtered and used in further analysis (solution A1).

*Granulate.* A mass of the five sachets corresponding to 10 mg of ibuprofen was weighed and then 10.0 mL of methanol was added and shaken for 30 min; the solution was filtered and used in further analysis (solution A2).

*Suspension.* The volume of the preparation corresponding to 10 mg of ibuprofen was pipeted, and then methanol was added to 10.0 mL and shaken for 30 min; the solution was used in further analysis (solution A3).

In the next step, 1.0 mL of solutions A1, A2 or A3 was made up with methanol in a 10.0 mL flask (solution B).

### Establishing TLC conditions

Standard solutions were applied on silica gel aluminium TLC $F_{254}$ plates, cut from 20 × 20 cm before use. Different volumes of the solutions (from 1 to 50 µL) were applied to the plates in triplicate, as 10 mm bands, 10 mm apart, 10 mm from the lower edge of the plate by means of a Linomat V automatic spray-on sample applicator equipped with a 100 µL syringe. The distance from the side of the plate to the first band was also 10 mm. The plates were then developed to different distances (from 8 to 18 cm) with experimentally selected mobile phases in a TLC chamber, previously saturated with the mobile phase vapor for 15 min at room temperature. For the distance of 13 cm the constituents were well separated in ~40 min (Figure 1). After development the TLC plates were dried in a current of air.

Densitometric scanning to locate spots on the chromatograms was performed with a TLC Scanner 3, equipped with the deuterium light source, in linear reflectance/absorbance mode, controlled by CATS 4 Software resident in the system. The slit dimensions were 8 × 0.45 mm, the scanning speed 20 mm/s and data resolution 100 µm per step. For individual constituents, the retardation factors $R_f$ were derived from the obtained densitograms. Analysis of UV absorption spectra, registered directly from chromatograms, recorded in the wavelength range of 200–400 nm, showed that maximum absorbance occurred at λ =

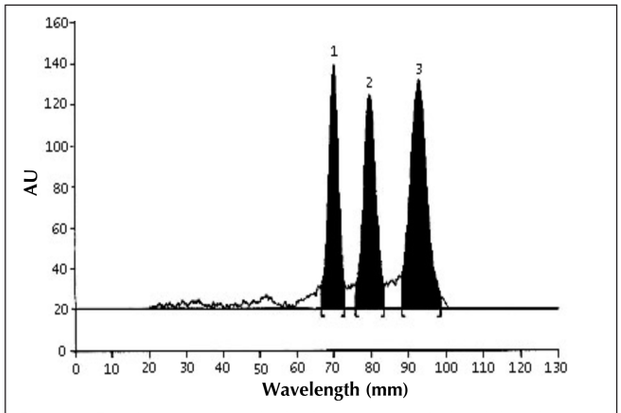


**Figure 1.** Densitogram of standard substances: 2-(4-isobutylphenyl)-propionamide (1, impurity A), 2-(4-isobutyrylphenyl)propionic acid (2, impurity B) and ibuprofen (3), obtained directly from chromatogram.

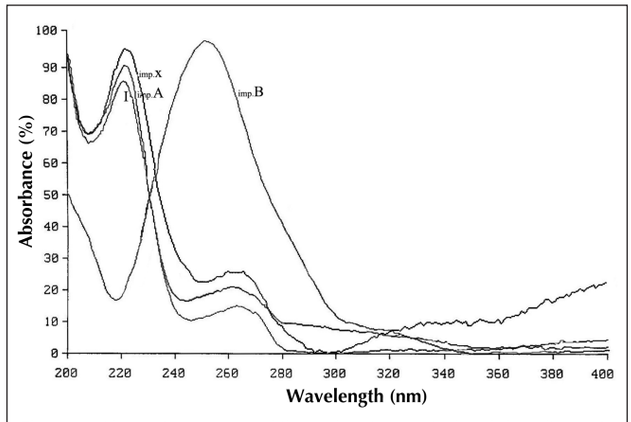


**Figure 2.** Absorption spectra of ibuprofen (I), 2-(4-isobutylphenyl)-propionamide (impurity A), 2-(4-isobutyrylphenyl)propionic acid (impurity B) and degradation product (impurity X), obtained directly from the TLC plate.

222 nm for ibuprofen and impurity A and λ = 252 nm for impurity B (Figure 2). The amount of the chromatographed compounds was determined from the intensity of diffusely reflected light.

### Quantitative analysis

The standard solutions at volume of 8 µL for ibuprofen, impurity A, and impurity B as well as 4 µL for sample solutions (solutions B) for determining active substance and 20 µL of sample solutions (solutions A) for determining impurities, were applied with the applicator to 9 × 13 cm plates. Chromatograms were developed to a distance of 13 cm with toluene–ethyl acetate–glacial acetic acid (17:13:1, v/v/v) as mobile phase. After development the plates were dried at room temperature. The densitometric measurements were made at 222 nm and 252 nm. If no additional peak of $R_f$ = 0.63 is registered on chromatograms at λ = 252 nm, analysis may be carried out at λ = 222 nm.

The concentration of constituents in preparation under examination were computed by comparing the peak areas for relevant standard and sample solutions. Three measurements were made for each determination. The mean values were taken as a final result. Received results with the statistical estimation were assembled in Table I. On the received densitograms there were no other peaks, beside the peaks originating from the active substance.

**Table I. Ibuprofen Determination in Preparations***

| Preparation (declared amount of ibuprofen) | Determined amount of ibuprofen Statistical analysis ($n$ = 6) | |
|---|---|---|
| Ibuprofen (200 mg in tablet) | $x_m$ = 207.55 mg<br>S = 4.04<br>µ = 207.55 ± 4.24 | $S_{x\,m}$ = 1.65<br>RSD = 1.95 |
| Nurofen (200 mg in tablet) | $x_m$ = 207.32 mg<br>S = 3.65<br>µ = 207.32 ± 3.83 | $S_{x\,m}$ = 1.49<br>RSD = 1.76 |
| Ibuprom (200 mg in tablet) | $x_m$ = 201.28 mg<br>S = 4.52<br>µ = 201.28 ± 4.76 | $S_{x\,m}$ = 1.85<br>RSD = 2.25 |
| Ibufen (100 mg in 5 mL of suspension) | $x_m$ = 99.35 mg<br>S = 2.13<br>µ = 99.35 ± 2.24 | $S_{x\,m}$ = 0.87<br>RSD = 2.14 |
| Nurofen (200 mg in 2.1 g of granulate) | $x_m$ = 201.50 mg<br>S = 3.71<br>µ = 201.50 ± 3.88 | $S_{x\,m}$ = 1.51<br>RSD = 1.84 |

* Arithmetic mean (xm); standard deviation (S); standard deviation of arithmetic mean (Sx m); confidence interval at 95% probability (µ); relative standard deviation percent (RSD).

**Table II. Validation Parameters of the Proposed TLC Method***

| Parameter | Ibuprofen | Impurity A | Impurity B | |
|---|---|---|---|---|
| λ (nm) | 222 | 222 | 222 | 252 |
| Rf | 0.72 | 0.54 | 0.63 | 0.63 |
| Linearity | P = 19.177 × c – 402.7<br>R = 0.99286 | P = 6.247 × c + 507.8<br>R = 0.99626 | P = 1284.0 × c + 2401.8<br>R = 0.99548 | P = 12.649 × c + 433.8<br>R = 0.99121 |
| LOD (µg/spot) | 0.24 | 0.13 | 0.15 | 0.17 |
| LOQ (µg/spot) | 0.72 | 0.40 | 0.46 | 0.51 |
| Precision | RSD = 1.30 | RSD = 1.62 | RSD = 1.44 | RSD = 2.06 |
| Intermediate precision | RSD = 1.85 | RSD = 2.04 | RSD = 1.98 | RSD = 2.33 |
| Accuracy | $x_m$ = 99.03%<br>RSD = 1.32 | $x_m$ = 96.75%<br>RSD = 2.62 | $x_m$ = 98.02%<br>RSD = 2.03 | $x_m$ = 97.56%<br>RSD = 2.45 |

* Peak area (P); concentration (c); correlation coefficient (R); arithmetic mean ($x_m$).

### Validation of the method

To confirm reliability of the results the method was validated (19). The results are presented in Table II.

The specificity of the method was determined by comparing the chromatograms obtained from the model solutions containing ibuprofen with those obtained from blank model solutions and analyzing them for peaks interfering with the detection of active substance. For methanol extracted solutions used for determination purposes, no additional spots were found, except for ibuprofen.

The linearity was checked on six solutions of various concentrations varying from 0.50 to 1.75 µg per spot for ibuprofen, from 6.00 to 16.00 µg per spot for impurity A and from 0.40 to 1.39 µg per spot for impurity B. The results were analyzed by employing the linear regression method.

Limits of detection (LOD) and quantitation (LOQ) were determined on the basic of the standard deviation of the response and slope of the straight lines, obtained from the equations: LOD = 3.3 × SD / a and LOQ = 10 × SD / a, where SD is the standard deviation of the response and a is the slope of the calibration curve.

The precision of the method was expressed as a consistence degree between the results of analyses carried out repeatedly. The tests were conducted for a model mixture solution containing ibuprofen, impurity A, and impurity B. The precision was estimated using peak areas of individual constituents and was evaluated by the standard deviation and relative standard deviation. Intermediate precision was determined by analyzing the same solutions on three different days over a period of one week.

Recovery was calculated by comparing the mean analytical results for the drug solutions with the theoretical value of the added weight of appropriate substance. The accuracy of the method was expressed in percentage of the recovery of added analyte within the range from 80% to 120% of relevant substances compared with the preparations under examination. The recovery (R[%]) was computed from the formula: $R\,[\%] = [(A - A_i) / A_i] \times 100\%$, where: A is the peak area [mm$^2$] obtained for the sample solution after adding a specified amount of analyte and $A_i$ is the peak area [mm$^2$] obtained before the analyte was added.

### Examination of ibuprofen stability in solutions

An effect of pH, temperature, and incubation time on stability of ibuprofen in solutions was investigated. For this purpose, weighed amounts of approximately 10 mg of ibuprofen were dissolved in 5 mL of hydrochloric acid or sodium hydroxide solution at a concentration ranging from 0.1 to 1.0 mol/L. The solutions were incubated at temperatures of 22, 37, and 70°C, while taking analyte samples at specified time intervals (from 7, 13–22 days). The samples were diluted with methanol (1:1, v/v) for quantita-

tive analysis. Internal normalization method to determine the percentage constituent concentration was used. The results obtained were used for kinetic and thermodynamic evaluation of the ibuprofen decomposition process, by determining the reaction rate constants $k$, half-life $t_{0.5}$ and the time $t_{0.1}$ in which the concentration of ibuprofen is reduced by 10%, as well as activation energy $E_a$, according to the kinetics of a first order reaction (20). The results are presented in Table III.

## Results and Discussion

A chromatographic–densitometric method was developed to simultaneously separate and determine the active substance of ibuprofen and its impurities. The conditions for separation of ibuprofen and analyzed impurities and the method for chromatographic identification were established by using appropriate standard solutions.

The application of the mobile phase consisting of: toluene–ethyl acetate–glacial acetic acid (17:13:1, v/v/v) provided good separation, and at the same time gave well developed peaks, important for good densitometric analysis. In addition to the values of retardation factors ($R_f$ = 0.74 for ibuprofen, $R_f$ = 0.54 for 2-[4-isobutylphenyl]propionamide [impurity A] and $R_f$ = 0.63 for 2-[4-isobutyrylphenyl]propionic acid [impurity B]) for individual constituents well developed and repeatable absorption spectra were recorded directly from chromatograms to aid in qualitative analysis (Figures 1,2).

**Table III. Kinetic and Thermodynamic Parameters Describing Degradation Process of Ibuprofen in Acidic Solutions***

| HCl conc. | Temp. | k ($h^{-1}$) | $t_{0.1}$ (h) | $t_{0.5}$ (h) |
|---|---|---|---|---|
| 1 mol/L | 22°C | $5.04 \times 10^{-4}$ | 209 | 1375 |
| | 37°C | $8.64 \times 10^{-4}$ | 122 | 802 |
| | 70°C | $2.53 \times 10^{-3}$ | 42 | 274 |
| 0.5 mol/L | 22°C | $2.03 \times 10^{-4}$ | 457 | 3414 |
| | 37°C | $4.33 \times 10^{-4}$ | 243 | 1600 |
| | 70°C | $1.46 \times 10^{-3}$ | 72 | 475 |
| | | $E_a$ (1 mol/L) = $2.87 \times 10^4$ | | |
| | | $E_a$ (0.5 mol/L) = $3.25 \times 10^4$ | | |

* Stability constant [k ($h^{-1}$)]; time, concentration will decrease about 10% [$t_{0.1}$ (h)]; time, concentration will decrease about 50% [$t_{0.5}$ (h)]; energy of activation [$E_a$ (J/mol × K)].

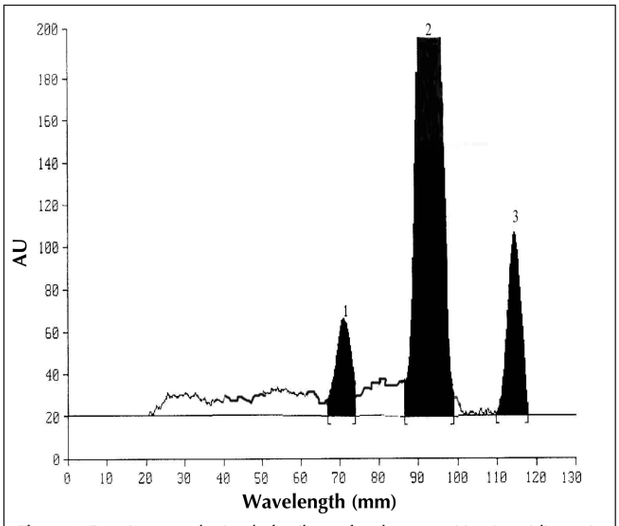


**Figure 3.** Densitogram obtained after ibuprofen decomposition in acidic environment (1 = impurity A; 2 = ibuprofen; 3 = impurity X).

The method presented above fulfills the principal requirements of good laboratory practices, both in the terms of quantitative analysis and determination of active substances and impurities. It is important that the method is characterized by good precision (RSD = 1.30–2.06%), high sensitivity (LOD from 0.13–0.72 µg per spot) and constituent detection, ranges of linearity and satisfactory recovery values varying from 96.8% to 99.0%. The results of determination of ibuprofen in the pharmaceutical products confirmed accuracy and good precision of the method. The obtained average contents of the active constituent are close to the declared content (Table I).

In the next part of the work the effect of pH in aqueous solutions on ibuprofen decomposition was analyzed. In addition to the ibuprofen peak, some additional peaks were observed on chromatograms depending on test conditions. In acidic environment, ibuprofen decomposes into two products ($R_{f(1)}$ = 0.54 and $R_{f(2)}$ = 0.90), while no additional peaks besides the peaks originating from ibuprofen were observed in basic solutions (Figure 3).

To identify the degradation products (impurity A and impurity X), $R_f$ values and UV spectra were recorded directly from chromatograms, and $^1$H NMR spectra were registered. Ibuprofen solution after hydrolysis in 1 mol/L HCl was separated on a chromatotron plate and after fraction separating, the solvent was evaporated and the products were identified with $^1$H NMR spectra comparative analysis. Because of very small amounts of degradation products, $^1$H NMR analysis did not bring any reliable results for the structure determination.

On the ground of $R_f$ values and absorption spectra, one can suppose that the spot of the ibuprofen's degradation product ($R_f$ = 0.54) originated from 2-[4-isobutylphenyl]propionamide (impurity A). This spot originated from acidic hydrolysis of the ibuprofen and corresponded to the appropriate spot from the ref-

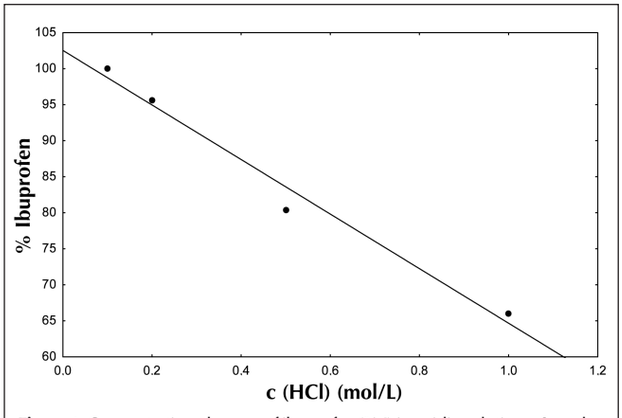


**Figure 4.** Concentration changes of ibuprofen (% I) in acidic solutions. Samples were incubated for 1 h at 70°C.

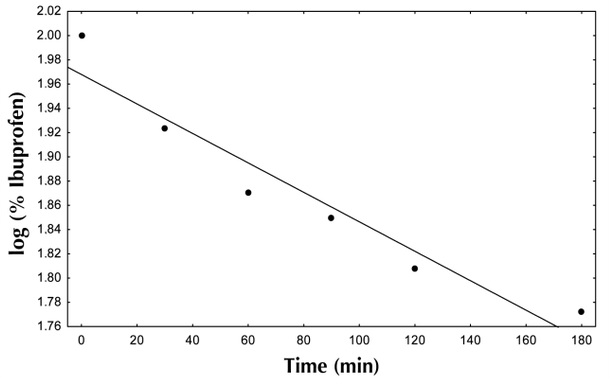


**Figure 5.** The relationship between concentration of ibuprofen (log % I) and incubation time. The studies were carried out in 1 mol/L HCl solution at 70°C.

erence substance. Unfortunately, it has not been possible to identify the spot with $R_f$ = 0.90 (impurity X) yet.

The concentrations of degradation products vary with hydrochloric acid concentration, temperature, and incubation time. Any effects of HCl concentration, temperature, and heating time on ibuprofen decomposition were examined.

Degradation of ibuprofen depends on the acid concentration (Figure 4). In acidic solutions at concentration of 0.2 mol/L and smaller, incubated at 70°C for 1 h, concentration change of active substance is less than 10%, contrary to experiments carried out in 0.5 and 1.0 mol/L solutions. The degradation process of ibuprofen depends on the incubation time. During longer incubation time, concentration of degradation products increases and concentration of active substance decreases. The relationship between log concentration of ibuprofen and time indicates that the degradation process of ibuprofen is consistent with kinetics for first-order reactions (Figure 5).

Temperature also influences on the stability of ibuprofen in acidic solutions. A temperature rise up to 70°C led to a decomposition of ibuprofen to ~65% in a week. Reaction rate constants for the degradation of ibuprofen increase with the increasing temperature and concentration of hydrochloric acid (Table III). Stability of ibuprofen described by kinetic parameters $t_{0.1}$ and $t_{0.5}$ is twice smaller in 1 mol/L hydrochloric acid than in 0.5 mol/L. Relatively high increase of the reaction rate constant $k$ with the temperature increasing corresponds to the obtained energy of activation $E_a$, and proves lower stability of ibuprofen in more acidic solutions. It should be pointed out that under described conditions two degradation products appear on chromatograms ($R_f$ = 0.54 and $R_f$ = 0.90) together with main peak of active substance ($R_f$ = 0.74).

## Conclusions

An easy and sensitive TLC chromatographic–densitometric method for simultaneous determination of ibuprofen and impurities, 2-[4-isobutyrylphenyl]propionic acid and 2-[4-isobutylphenyl]propionamide was developed. This TLC-densitometric method can be regarded as an alternative to the more widely used HPLC, because sample preparation is simpler and there is a possibility of multi-sample analysis which reduces analysis cost and time for individual samples. The proposed method is characterized by high specificity, and also accurate and precise enough to be successfully adopted as an alternative to the existing methods for evaluation of drugs in pharmaceutical preparations and quality control.

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