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An innovative approach to the analysis of 3-[4-(2-methylpropyl)phenyl]propanoic acid as an impurity of ibuprofen on a carbon-coated zirconia stationary phase

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ABSTRACT

3-[4-(2-Methylpropyl)phenyl]propanoic acid has been introduced as impurity F to the European Pharmacopoeia in its Supplement 4.2. In contrast to other impurities, which are evaluated by HPLC, the content of impurity F is determined by gas chromatography after previous derivatization. Thus a novel reversed-phase HPLC method was developed to simplify the evaluation of pharmacopoeial impurity F of ibuprofen. Favourable properties of zirconia stationary phases were employed for this purpose. The HPLC separation was achieved on a Zr-CARB column (150 mm × 4.6 mm i.d., 5 μm) using the mobile phase acetonitrile–phosphate buffer (pH 3.5, 25 mM) (38:62, v/v), temperature 80 °C and the flow rate 1.2 ml min^{-1}. The fluorescence detection was employed to enhance the sensitivity of the method. Optimal detection parameters were chosen on the basis of fluorescence spectra of the analytes. The excitation and emission wavelengths were 220 nm and 285 nm, respectively. The analysis was completed within 25 min. The subsequent validation of the method confirmed the applicability of method for the analytical assay of impurity F.

1. Introduction

Perfectly mastered preparation technology of silica particles, wide variety of commercially produced stationary phases, satisfactory separation of most types of analytes and high efficiency are the main reasons why silica-based columns represent the most important instrument for chromatographic purposes. The solutes are interacting with RP-SiO_2 support predominantly via hydrophobic interactions. Nevertheless even the highly purified silica packing has not eliminated complications yielding from residual silanol groups. This inconvenience counts obviously for basic analytes [1].

In parallel to improvement of silica materials, the effort of researchers has been focused on the investigation of other types of columns with longer durability and better reliability regardless of mobile phase composition and acid–base character of analytes. Attractive chemical properties of some metal oxide stationary phases have been published previously [2,3]. Aluminia, zirconia and titania have been utilized as promising materials to provide superior chromatographic support with respect to analogous silica ones. Nowadays zirconia represents the most investigated material for separation purposes [2,4]. Except for the superb stability, the unique surface chemistry is another point of interest. Analyte–stationary phase interactions are influenced (besides reversed-phase contribution) also by ion-exchange and ligand-exchange mechanism. The character of ion-exchanger is dependent on pH. Zirconia acts as an anex in acidic and neutral solutions but it works as a catex in alkaline solutions. The existence of Lewis acid sites on the surface of zirconia is responsible for the ligand-exchange ability [3,5]. As a consequence of these interactions mixed mode retention is observed [6] and thus altered selectivity gives more space for a method development, especially if analytes differ slightly from each other.

Chromatographic separations are predominantly performed in a reversed-phase mode. Unlike silica gel the zirconia is not suitable for a silanization due to considerably lower stability of Zr–O–Si–R bond than Si–O–Si–R bond. A preparation of bonded phase via silanization is also hindered by the presence of bridged form of hydroxyls on the zirconia surface. This type is unsuitable for silanization reaction due to its structure and basicity [3].

Commercially available zirconia reversed-phase stationary phases are manufactured by introducing of polymer or carbon layer onto the zirconia surface.

Polybutadiene (Zr-PBD)- and polystyrene (Zr-PS)-coated zirconia represent alternative materials to traditional ODS columns. Both are less hydrophobic than typical C18-silica phases [7]. A polystyrene column serves as a useful option for analytes that cannot be resolved on Zr-PBD. In addition, Zr-PS offers interesting selectivity for aromatic compounds thanks to its aromatic functionality [8].

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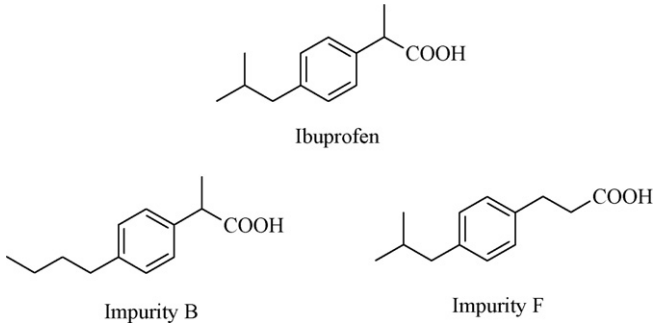

Fig. 1. Chemical structures of ibuprofen and its impurities B and F.

Higher retention, especially for polar and highly aromatic analytes, is reached on a zirconia surface with a thin layer of pure graphitized carbon (Zr-Carb). Compared to typical carbon columns limited by high fragility [1], zirconia bed offers evidently ruggedness, extended column lifetime and reproducibility. It was found that these sorbents show shape specificity and thus their retention and selectivity are dramatically influenced [9]. This type of columns appears optimal for separation of geometrical isomers and diastereomers [10].

A surface with C18 chains (Zr-C18) is obtained through chemical modification of carbon layer anchored on the zirconia surface. Respecting the retention behaviour and selectivity, Zr-C18 columns are the compromise between Zr-PBD and Zr-Carb packing [3,7].

1.1. Ibuprofen (R,S)-2-[4-(2-methylpropyl)phenyl]propanoic acid

Ibuprofen (Ibu) belongs to a broad and heterogeneous group of non-steroidal antiinflammatory agents. Variety of indications, including the treatment of wide spectra of clinical symptoms, such as pain, inflammation, arthritis, fever, etc., are in consistency with its antipyretic, analgesic, antiinflammatory and antiaggregatory properties. Ibu disposes of improved gastrointestinal tolerance than pyrazolone derivatives, salicylates and indomethacin. Generally, Ibu is available in a number of dosage forms including oral, rectal and topical ways of administration. It represents a very efficient over the counter drug with a proper safety profile and a quite low incidence of adverse effects [11].

The approaches to the evaluation of ibuprofen and its impurities differ among worldwide-accepted pharmacopoeias.

The European Pharmacopoeia (Ph. Eur.) as well as The British Pharmacopoeia exploit a gradient LC method using an octadecyl silica gel column (except for impurity F) in the purity evaluation of Ibu. The separation power of the system is tested by the separation of the principal peak (Ibu) and its chain isomer impurity B, see Fig. 1, where peak-to-valley ratio has to be at least 1.5 [12,13].

Impurity B, a manufacture process residue [14], was of great importance in the previous issue of the European Pharmacopoeia. Its content in the substance was limited to 0.3% and was calculated relatively to the standard solution of impurity B. The content of all other individual impurities was related to the peak area of Ibu standard solution [15]. Nevertheless, the current European Pharmacopoeia has not already required the individual assay of impurity B. It is, together with other 13 impurities, involved in a group of detectable impurities. Their presence is dependent on a manufacture process of Ibu and the content of each impurity has not to be more than 0.05%. The amount of 0.15% is allowed for impurities labelled as A, J and N, respectively [12].

However, special attention is paid to the impurity F. The first mention of impurity F appeared in the Supplement 4.2 of Ph. Eur. [16]. The approach to analysis of this substance consists of methylation reaction (see Fig. 2) followed by a gas chromatography method [12]. After the methylation procedure (100 °C, 20 min) the product is cooled and dried under a stream of nitrogen. The residue is dissolved in ethyl acetate and analyzed by GC-FID. Relative retention of impurity F, related to ibuprofen, has to be about 1.5. The amount of impurity F has not to be higher than 0.1% [12].

The US Pharmacopoeia describes a similar RP-HPLC method for the estimation of related substances. However chromatographic data are processed according to the normalisation method. The area of any individual peak must not exceed 0.3% and the limit for the sum of all individual impurities is 1.0%. Unlike Ph. Eur., a degradation product of Ibu—4-isobutylacetophenone is the only specified impurity [17]. The main differences between Ph. Eur. and USP are summarized in Table 1.

The Japanese Pharmacopoeia does not define any related compounds and uses a TLC method for the purity analysis of Ibu. The amount of individual impurity is related to an Ibu solution and must not exceed 1% [18].

Several authors have already dealt with the separation of Ibu and its related compounds on silica-based [19–23] as well as zirconia-based [24,25] columns, but an HPLC method enabling analysis of the recently introduced pharmacopoeial impurity F has not been published so far. Since both substances (i.e. ibuprofen and impurity F) are positional isomers, the differences in their physicochemical properties are practically negligible. Moreover, impurity B also possesses very similar properties to both mentioned compounds as shown in Table 2 [26]. Thus the separation of these analytes from each other requires a chromatographic system with an enhanced selectivity.

Our previous experience with separation of Ibu on zirconia-based stationary phases [24,25] encouraged us to focus on feasibility to separate Ibu and its impurity F using a HPLC system and avoid a quite tough GC method. With respect to the valid European Pharmacopoeia our objective was to develop a simple HPLC assay in this manner. The maintenance of some system suitability qualifications as well as the enhancement of detection sensitivity was also an important step during this work. Naturally, the verifying of the suggested method was of great importance in conclusion.

2. Experimental

2.1. Instruments

All chromatographic measurements were performed on a Shimadzu system, equipped with the system controller SCL—$10A_{VP}$, fluorescence detector RF—$10A_{XL}$, LC pump—$10AD_{VP}$, autoinjector SIL—$10AD_{VP}$, column oven CTO—$10AS_{VP}$, degasser DGU—14A,

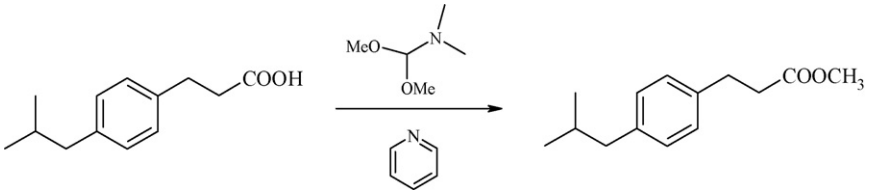

Fig. 2. Reaction schema of impurity F derivatization.

Table 1
Pharmacopoeial approaches to the impurity analysis of ibuprofen according to the Ph. Eur. and USP.

Methods	Ph. Eur. (HPLC; GC)	USP (HPLC)
HPLC method	Gradient reversed phase	Isocratic reversed phase
Stationary phase	ODS	ODS
Column size	150 mm × 4.6 mm i.d.	150 mm × 4.0 mm i.d.
Mobile phase composition	Water–phosphoric acid 85%–acetonitrile (660:0.5:340, v/v/v)	Water (pH 2.5 adjusted with phosphoric acid)–acetonitrile (1340:680, v/v)
Detection	UV 214 nm	UV 214 nm
Column temperature	Ambient	30 °C
Quantification method	Regarding to ibuprofen peak 0.1%	Normalization percentage
Limit for specified impurities	0.15% (counts for Imp *A*, *J*, *N*[a])	0.1% (counts for isobutylacetophenone)
Limit for unspecified impurities	0.05%	0.3%
Limit for the sum of all impurities	0.2%	1%
GC method		No
Stationary phase	Macrogol 20000 (film thickness 2 pm)	
Column size	25 m × 0.53 mm i.d.	
Column temperature	Isothermic 150 °C	
Injection/detection	200 °C/flame ionisation 250 °C	
Impurity F	After methylation reaction	
Quantification method	Regarding to Imp F peak 0.1%	
Limit	0.1%	

[a] Imp *A* = (2*RS*)-2-[3-(2-methylpropyl)phenyl]propanoic acid Imp *J* = (2*RS*)-2-[4-(2-methylpropanoyl)phenyl]propanoic acid Imp *N* = (2*RS*)-2-(4-ethylphenyl)propanoic acid.

low pressure module FCV—10AL$_{VP}$ and computer-based chromatographic software Class-VP, ver. 6.12 Shimadzu (Tokyo, Japan). The fluorometer Aminco-Bowman Series 2 Luminescence Spectrometer (Madison, USA) was utilized for the measurement of fluorescence spectra. The UV-VIS spectrometer UV2401PC Shimadzu (Tokyo, Japan) was used for measurement of UV-spectra.

2.2. Chromatographic columns

During experimental work following chromatographic columns were tested: ZirChrom-CARB, 150 mm × 4.6 mm i.d., particle size 5 μm, DiamondBond-C18, 150 mm × 4.6 mm i.d., particle size 5 μm, ZirChrom Separations (Anoka, USA) and Discovery Zr-PS, 150 mm × 4.6 mm i.d., particle size 5 μm, Sigma–Aldrich (Schnelldorf, Germany) and SiO_2-C18 column, 150 mm × 3.0 mm i.d., particle size 7 μm, Tessek (Prague, Czech Republic).

2.3. Chemicals

Ibuprofen Sigma (St. Louis, MO, USA); 3-[4-(2-methylpropyl) phenyl]propanoic acid (impurity F) and the solution 0.06 mg ml^{-1} of (2*RS*)-2-(4-butylphenyl)propanoic acid (impurity B) were purchased from Council of Europe, European Directorate for the Quality Control of Medicines (Strasbourg, France). Solvents used for preparing mobile phases were all HPLC-grade. The other chemicals were analytical-grade or better and were obtained from common commercial sources.

2.4. Sample preparation

The mixture of acetonitrile–water (50:50, v/v) was used as a diluent for the sample preparation. A standard solution of Ibu (2 mg ml^{-1}) was prepared by accurately weighing 500.0 mg of Ibu into 250 ml volumetric flask, dissolving and diluting to the mark. A stock solution of impurity F was prepared at the concentration 1 mg ml^{-1}. A standard solution of impurity F was prepared by diluting the stock solution with the diluent to the concentration of 2 μg ml^{-1}.

A model solution (containing Ibu and pharmacopoeial limit of impurity B and F) used for the assessment of system suitability was prepared by adding 20 μl of impurity F stock solution and 100 μl of impurity B solution into 10 ml volumetric flask and diluting to the mark with Ibu standard solution. This solution corresponds to 0.1% amount of impurity F and 0.03% amount of impurity B.

The injection volume of all samples was 10 μl.

2.5. Buffer preparation

Buffers were prepared by dissolving appropriate amount of ammonium dihydrogenphosphate in water with resulting molar

Table 2
Comparison of physicochemical characteristics of ibuprofen, impurity F and impurity B.

Property	Ibu	Impurity F	Impurity B	*Condition*
	Value			
H donors	1	1	1	
H acceptors	2	2	2	
Molecular Weight	206.28	206.28	206.28	
log *P*	3.722 ± 0.227	3.709 ± 0.207	3.906 ± 0.222	
log *D*	3.72	3.71	3.91	pH 1
log *D*	3.58	3.63	3.77	pH 4
log *D*	1.16	1.40	1.37	pH 7
log *D*	0.36	0.53	0.56	pH 8
log *D*	−0.02	−0.03	0.16	pH 10
pK_a	4.41 ± 0.10	4.68 ± 0.10	4.43 ± 0.10	Most acidic
Molar solubility	Sparingly soluble	Sparingly soluble	Sparingly soluble	pH 1
Molar solubility	Sparingly soluble	Sparingly soluble	Sparingly soluble	pH 4
Molar solubility	Slightly Soluble	Slightly soluble	Soluble	pH 7
Molar solubility	Soluble	Soluble	Soluble	pH 8
Molar solubility	Very soluble	Very soluble	Very soluble	pH 10

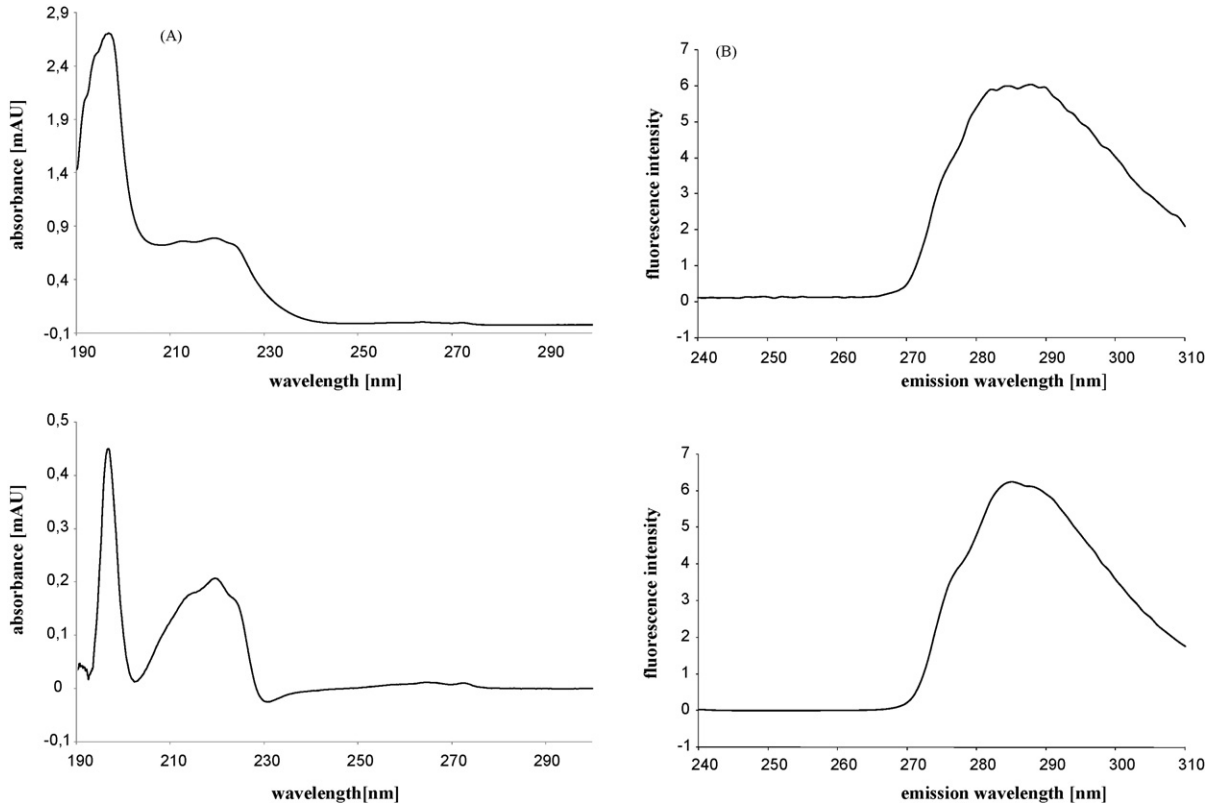

Fig. 3. UV (A) and fluorescence (B) spectra of Ibu (above) and impurity F (below).

concentration of 25 mM. The proper pH value was adjusted by addition of diluted phosphoric acid or ammonium hydroxide.

3. Results and discussion

As mentioned above the evaluation of 3-[4-(2-methylpropyl) phenyl]propanoic acid as the impurity F of Ibu was introduced into the Ph. Eur. in 2002. The quantification of this compound is accomplished by GC analysis after the methylation of carboxylic group.

Our attempt to analyze the impurity F by the pharmacopoeial method failed. According to our experience the method suffers from the lack of selectivity and sensitivity as well.

Detection: Although the UV spectrum of impurity F is similar to the UV spectrum of Ibu, see Fig. 3A, the response of a UV detector to impurity F in concentrations corresponding 0.1% (i.e. 2 $\mu g\,ml^{-1}$) is insufficient and thus the lack of sensitivity is observed. Nevertheless, this drawback could be overcome by using fluorimetric detection. The emission radiation of Ibu and its related compounds reached maximum intensity at wavelengths around 285 nm when their excitation had been performed at 220 nm, see Fig. 3B. So to enhance sensitivity to impurity F the compounds were detected under following conditions—excitation: 220 nm; emission: 285 nm.

Stationary phase selection: The chromatographic behaviour of Ibu, impurity B and impurity F was very similar on the conventional C18 sorbent, thus low selectivity towards these analytes was observed. The peak due to impurity F eluates before the peak due to impurity B, moreover this pair was not completely resolved. It is important to take into account that separation of Ibu and impurity B is a critical step of the pharmacopoeial procedure, and it is involved in the system suitability test. Hence the detection of impurity F is impossible because it is overlapped by the principal peak due to Ibu.

On the basis of our previous experience [24,25] the exploitation of zirconia stationary phases was tested to solve this problem of selectivity. Three RP-zirconia stationary phases were tested: zirconia modified with polystyrene, carbon layer and carbon layer with chemically attached C18 chains, respectively.

The Zr-C18 column was tested as an alternative to the silica-based C18 column. However it behaved similarly to ODS phase and practically no improvement in separation was achieved. The retention of impurity F was slightly stronger but insufficient to be separated from impurity B, see Fig. 4. Probably, the separation is predominantly influenced by the presence of C18 chains and that is why the similar results were observed on both columns.

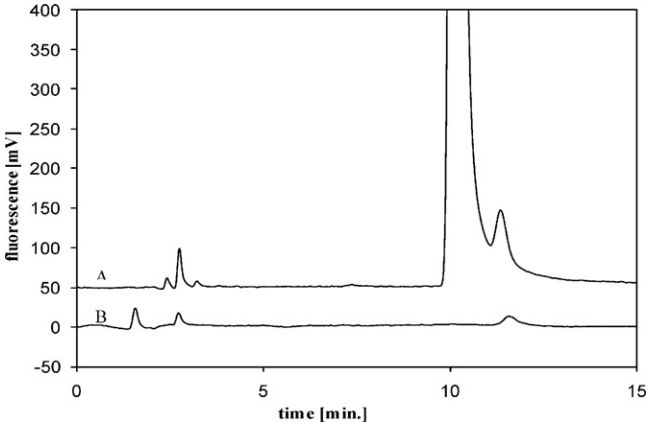

Fig. 4. (A) Separation of Ibu and impurity B and (B) chromatogram of impurity F on DiamondBond-C18 column. *Chromatographic conditions*: mobile phase ACN–phosphate buffer (pH 2.1, 25 mM) (40:60, v/v); fluorescence detection 220 nm (excitation) and 285 nm (emission); flow rate 1 ml min^{-1}; temperature 80 °C.

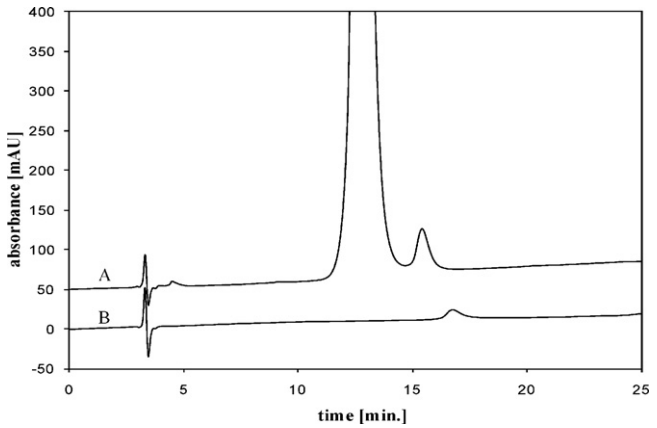

Fig. 5. (A) Separation of Ibu and impurity B and (B) chromatogram of impurity F on Zr-PS column. *Chromatographic conditions*: mobile phase MeOH–phosphate buffer (pH 4.5, 50 mM)–THF (21:74:5, v/v/v); UV detection 219 nm; flow rate 0.5 ml min^{-1}; temperature 60 °C.

A PS-ZrO_2 was chosen in view of its ability to provide selectivity for the separation of structural isomers which differ in the position of phenyl groups [8]. In our previous work, better separation of Ibu and impurity B was achieved on the Zr-PS column than on the silica gel C18 column [24]. Therefore, the same system was used also for separation of impurity F from Ibu and impurity B. Although this stationary phase is less hydrophobic as conventional ODS phases quite promising results were obtained. Both related compounds were well separated from Ibu (Fig. 5) and an inverse elution order was observed in comparison to the results achieved on a silica-based C18 column. However, better resolution between these impurities could be observed on the third column.

The Zr-Carb stationary phase possesses different selectivity than C18 phases [9] and it is recommended in cases when an ODS column does not work [7]. Carbon-clad zirconia differs from alkyl-bonded phases in following characteristics: it is more hydrophobic, possesses better selectivity to geometrical isomers and polar solutes are retained also through electronic (π–π)-interaction. The retention mechanism is thought to be more influenced by the solute polarizability, dipolarity and shape than on aliphatic phases. Since the retention process is very sensitive to the shape of a solute, carbon surfaces are much more selective for the separation of geometrical isomers than typical bonded phases with alkyl chains or polymer-coated metal oxides [9,10]. The best separation of all analytes was reached on this column, see Fig. 6. When taking into account that the physicochemical properties of all compounds are practically identical, the sensitivity to molecule shape seemed to be responsible for the sufficient separation of Ibu from its related compounds. The improved resolution between Ibu and impurity F is especially notable.

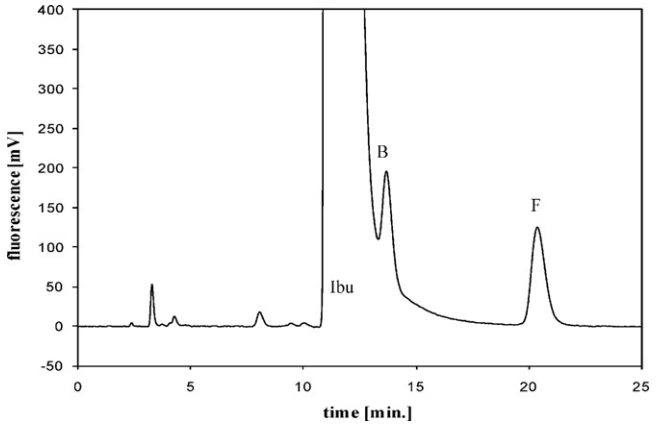

Fig. 6. Separation of Ibu and impurities B and F on ZirChrom-CARB column (model solution) under optimal chromatographic conditions: mobile phase ACN–phosphate buffer (pH 3.5, 25 mM) (38:62, v/v); fluorescence detection 220 nm (excitation) and 285 nm (emission); flow rate 1.2 ml min^{-1}; temperature 80 °C.

Method optimization: After selection of the column and detection technique, the chromatographic system was optimized in terms of sufficient resolution between Ibu and impurity B (system suitability test) and reasonable analysis time. The influence of different organic modifiers (methanol and acetonitrile), pH of mobile phase and buffer type on separation were investigated.

As expected, better performance was gained by using acetonitrile as the organic part of the mobile phase. The substitution of acetonitrile with methanol led to worse peak shape and peak broadening. Because of the acid character of all analytes the influence of pH on the retention in the range 2–4 was practically minimal. The samples are almost non-ionised with regard to their pK_a values (see Table 2).

The retention was controlled predominantly by reversed-phase mechanism. If the pH of mobile phase got over pK_a of the analytes, a dramatic decrease of retention was observed due to repulsion forces between charged analytes and negatively charged surface of the stationary phase [2,5,6,27]. Unfortunately, the selectivity of the column decreased as well—at pH 6 all peaks were overlapped and eluted with the dead volume.

It is well known, that the chromatographic behaviour on zirconia-based stationary phases is also considerably influenced by the buffer type. This phenomenon is caused by the existence of hard Lewis acid sites on the surface of zirconia. Their presence allows Lewis base functional groups to interact with the surface. Such a Lewis acid–base interaction result in an especially slow desorption kinetics, which causes the formation of broad and tailed peaks. However, when a strongly competing Lewis base is deliberately added to the eluent at sufficiently high concentration, the accessible Zr(IV) sites are dynamically blocked [6,27]. We tested phosphate and acetate anions as an additive to the mobile phase. No obvious influence of the acetate buffer on selectivity in comparison with the phosphate buffer was found, only the peak shape was slightly worse. Fluoride testing was avoided because the mobile phase pH was mostly below 4.0. Under these conditions hydrofluoric acid is formed and it is very corrosive towards HPLC equipment [7,28].

The optimal chromatographic conditions were following—stationary phase: ZirChrom-CARB, 150 mm × 4.6 mm i.d., particle size 5 μm; mobile phase: ACN—phosphate buffer (pH 3.5, 25 mM) (38:62, v/v); fluorescence detector set at 220 nm (excitation wavelength) and 285 nm (emission wavelength); flow rate 1.2 ml min^{-1} and column temperature 80 °C.

Under these conditions a sufficient separation of analytes was achieved—Ibu = 12 min, impurity *B* = 14 min and impurity *F* = 21 min, see Fig. 6. During optimization of chromatographic conditions it was found that the analysis is not affected by small changes of experimental conditions. The content of acetonitrile might be adjusted in order to meet the sufficient resolution between Ibu and impurity B. As far as the stability of the sample is concerned it was found that the samples are stable in autosampler (15 °C) for at least 48 h. The applicability of developed method with regard to sensitivity, preciseness and repeatability was tested afterwards [29].

3.1. Validation procedure

Linearity: The linear dependence of the peak area on the concentration of impurity F was tested on 11 levels within the concentration range from 2400 to 2 ng ml^{-1} (i.e. 120–0.1% of the allowed amount). Each concentration level was injected four times.

Based on experimental data linear dependence was found in the range of 2400–100 ng ml^{-1}. The equation of regression was $y = 1.97 \times 10^{-5}x - 1.13$, and correlation coefficient, r^2, was equal to 0.9986.

Limits of detection and quantification: The limit of detection (LOD) is defined as a concentration which gives a treble signal of noise and the limit of quantification (LOQ) is defined as a concentration which gives a signal about 10 times higher than a baseline noise. The intensity of the noise was obtained from the chromatogram of Ibu (2 mg ml^{-1}) on the descending part of the peak. The peak heights of impurity F were obtained by analysis of samples containing Ibu (2 mg ml^{-1}) and impurity F in concentration 200, 100 and 50 ng ml^{-1}, respectively. Afterwards both limits were calculated on the basis of these data. The obtained values are as follows: LOD = 46 ng ml^{-1} (2.3% of the admissible amount) and LOQ = 153 ng ml^{-1} (7.7% of the admissible amount). It can be seen that the presence of Ibu slightly influences the value of LOQ in comparison to results achieved in linearity testing.

Response factor: The average responses of fluorescence detector to 2 μg ml^{-1} solutions of impurities F and B were found and were divided by the response of equally concentrated solution of Ibu (see Fig. 7). Calculated values for impurity F and impurity B are 4.79 and 8.37, respectively. From these response factors it is obvious that both impurities have higher fluorescence rate than Ibu alone. Hence normalisation method for the calculation of impurity F content that is applied in the pharmacopoeial GC method [12] would be in our case inappropriate.

Accuracy: The accuracy of the method was confirmed by determination of average recoveries from the samples through the method of external standard. Considering the elution of peak due to impurity F on the tailing part of peak of Ibu, not only 100% recovery level was tested. The final concentration levels of impurity F were 120%, 100%, 50% and 10%. Two weights were prepared from each concentration and four injections were carried out from every sample. All measured recoveries are shown in Table 3. The obtained results are reflecting sufficient accuracy.

Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. The estimation of repeatability was accomplished utilizing six samples of impurity F standard solution, each one injected four times. Relative standard deviations were calculated and are displayed in Table 3. Since the concentration level of impurity F is three orders lower than concentration level of principal peak due to Ibu, the value R.S.D. = 1.30 is very reasonable, and we can claim a good precision

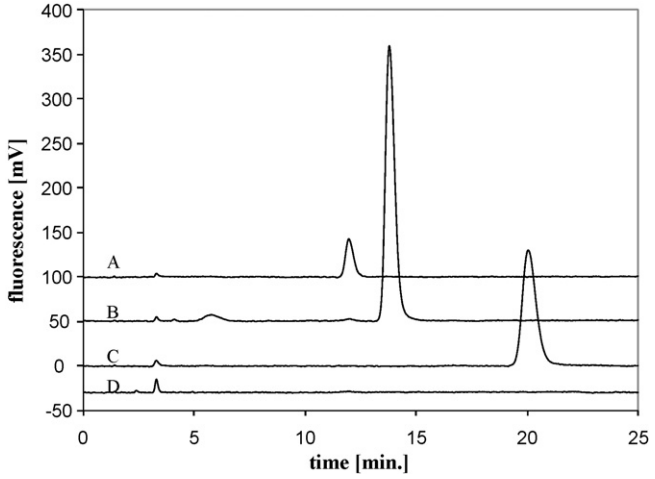

Fig. 7. Selectivity of the method. (A) Ibu (2 μg ml^{-1}); (B) impurity B (2 μg ml^{-1}); (C) impurity F (2 μg ml^{-1}); (D) ACN–water (50:50, v/v). All chromatograms were obtained under optimal conditions.

Table 3
Accuracy and repeatability of the method of impurity F determination.

Sample	Recovery [%]			
	100% level	120% level	50% level	10% level
Accuracy				
1	102.08	122.33	50.23	9.98
2	101.84	120.19	49.77	9.91
Average	101.96	121.26	50.00	9.94
Sample			R.S.D. [%]	
Repeatability				
3			0.57	
4			0.67	
5			0.46	
6			0.97	
7			0.38	
8			0.21	
Total			1.30	

of the method. At the same time the repeatability of injection was measured utilizing five injections of impurity F standard solution. Obtained value of R.S.D. = 0.58 documents that autosampler was suitable for validation.

Selectivity: The selectivity of the chromatographic system is displayed in Fig. 7. Pharmacopoeial requirement of minimal peak-to-valley ratio between Ibu and impurity B has been reached as well. Time of a single analysis of about 25 min is needed to achieve satisfactory resolution among the separated analytes with regard to the overwhelming peak of Ibu.

4. Conclusion

Zirconia-based stationary phase coated with graphitized carbon exhibits a number of advantages in comparison with traditional carbon column packings. This type of column offers more space for the method development utilizing both whole pH range and wider range of separation temperatures. On the basis of our experimental work it was confirmed that it is helpful and well suited for the separation of impurities that differ from each other only by an arrangement of atoms.

In European Pharmacopoeia the content of 3-[4-(2-methylpropyl)phenyl]propanoic acid as newly quantified impurity of ibuprofen has been determined through a quite tough GC method after derivatization. As an option, a simple isocratic HPLC separation method for evaluation of its content in an ibuprofen substance has been developed in our laboratory. The detection limit 46 ng ml^{-1} (i.e. 2.3% of the admissible amount) is considerably below the limit concentration 2 μg ml^{-1} of impurity F. Even though the chemical structure and physicochemical properties of Ibu, impurity B and impurity F are almost identical, the successful separation was accomplished within approximately 25 min. Impurity B was included to test the system suitability and sufficient peak-to-valley ratio was achieved. In conclusion, the developed method seems to be appropriate for the routine evaluation of impurity F.

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