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Use of the zirconia-based stationary phase for separation of ibuprofen and its impurities

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Abstract

A new reversed-phase liquid chromatographic method using zirconia-based stationary phase was developed for determination of ibuprofen, its related compounds and its main degradation products. The chromatographic separation was successfully achieved on the Discovery® Zr-PS column (150 mm × 4.6 mm i.d., 5 μm), using a mobile phase methanol–phosphate buffer (pH 4.5; 0.05 M)–tetrahydrofurane (21:74:5, v/v/v) and the flow rate 0.5 ml min^{-1}. The UV detection was performed in dual wavelength mode (219 and 258 nm) to detect all compounds of interest. The column temperature was set on 60 °C to shorten the analysis time and improve the peak symmetry. The method is simple, rapid and cuts down the amount of hazardous waste produced in the analysis. The assay is completed within 22 minutes.

Keywords: Zirconia-based stationary phase; Ibuprofen; Impurities

1. Introduction

1.1. Zirconia-based analytical columns

Nowadays most analytes are processed on alkyl silane bonded silica-based stationary phases. Solutes are bonded predominately by hydrophobic (reversed-phase) interactions with the bonded site. However, the presence of residual silanol groups on silica's surface greatly complicates the retention process especially for basic drugs [1].

Zirconia is a material with many unique properties which make it attractive as a chromatographic support, notably its excellent chemical stability and unique surface chemistry [2]. Zirconia due to its superb chemical and thermal stability is one of the major alternatives to a silica [2–5]. It is an amphoteric material with anion-exchange properties in neutral and acidic solutions and cation-exchange properties in alkaline solutions [2]. Numerous studies have shown that zirconia-based materials can be used for separation of nonpolar and polar solutes over the pH range 0–14 and temperatures up to 200 °C [1,6–10]. In contradistinction to the behaviour of the silanol groups, Zr(IV) sites (hard Lewis acids) on zirconia cause hard Lewis base analytes ($R\text{-}SO_3^-$, $R\text{-}PO_3^-$, $R\text{-}COO^-$, etc.) to absorb quite tenaciously [1]. Such Lewis acid–base interactions are characterised by especially slow desorption kinetics, which can broaden and tail peaks. However when a strongly competing Lewis base (PO_4^{3-}, F^-, carboxylates) is deliberately added to the eluent in sufficiently high concentration, the accessible Zr(IV) sites are dynamically blocked by absorption of the eluent. Addition of the hard Lewis base to the eluent thereby greatly improves the peak shape of the analytes. Polybutadiene-coated zirconia (PBD-ZrO_2) has been the most studied zirconia bonded reversed-phase material today [1,9–13].

According to the paper reported by Zhao and Carr [14] another type of zirconia coated stationary phase seems to have more favourable properties than PBD-ZrO_2. Polystyrene coated-zirconia (PS-ZrO_2) does exhibit different selectivity than PBD-ZrO_2 or octadecyl-bonded silica (C18-SiO_2) to-

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wards a variety of analytes and it is a unique, selective stationary phase, which can provide effective separations for many compounds. In addition, PS-ZrO_2 exhibits much greater selectivity than does PBD-ZrO_2 for the separation of structural isomers which differ in the position of phenyl group. In general, even though the carbon content of PS-ZrO_2 is much lower than that of conventional reversed-phase material, such as C18-SiO_2, polar analytes display sufficient retention. In several examples comparable or better resolution were found in much shorter analysis time. PS-ZrO_2 exhibits good mass transfer characteristics, furthermore the material is stable at extreme pH (1–13) and at temperatures as high as 160 °C [14].

1.2. Ibuprofen

Ibuprofen – (*R*,*S*)-2-(4-isobutylphenyl)propionic acid – was introduced in the late sixties as a safe non-steroidal anti-inflammatory drug. Ibuprofen (Ibu) is for its analgesic, antipyretic and platelet antiaggregatory properties used in a treatment of rheumatoic arthritis, osteoarthritis, fever, pain, migraine and dysmenorrhoea. Ibu is better tolerated than aspirin, indomethacin and pyrazolonic derivatives. In addition, it is well absorbed from gastrointestinal tract following oral or rectal administration. There is also some absorption following topical application to the skin [15–17].

Ibu is widely used as a therapeutic drug and several HPLC [18–22], ITP [23], MEKC [24–26] or SPE-UV [27] methods for its determination in pharmaceutical preparations, as well in the biological material [28] have been described in the literature.

The approach to quality control of Ibu differs in the United States and in the European countries. The US Pharmacopoeia describes a RP-HPLC method for impurities using a 150 mm × 4.0 mm i.d., column, mixture of water (pH 2.5)–acetonitrile (1340:680, v/v) as an eluent, UV detection at 214 nm and the temperature 30 °C. The area of an individual secondary peak, which appears in the test sample, should not exceed 0.3% and the sum of secondary peaks must not be higher than 1%. The compounds are not specified. A special attention is paid to a content of 4-isobutylacetophenone (Ibap) [29]. It is well known that Ibap causes adverse effect in the central nervous system and presents high dermal adsorption [19]. The assay is carried out under different conditions than the first chromatography 250 mm × 4.6 mm i.d. column, a mixture of 1% chloroacetic acid solution (pH 3.0)–acetonitrile (40:60, v/v) as an eluent, UV detection at 254 nm, ambient temperature. The amount of 4-isobutylacetophenone is not higher than 0.1% [29].

The British Pharmacopoeia (BP) describes a similar HPLC method for related substances of Ibu in raw material and in pharmaceuticals like the USP. The BP specifies five substances as possible impurities and indicates the importance of control of 2-(4-*n*-butylphenyl)propionic acid (Bppa) in raw material and pharmaceuticals (except oral suspension), which content must not exceed 0.3% and the area corresponding to the sum of secondary peaks must be lower than 0.7% of the Ibu peak. The amount of 4-isobutylacetophenone in oral suspension is controlled (0.25%) [30].

The European Pharmacopoeia has brought into practice a gradient method for quality control of Ibu and has described potential impurities A–R. The substances A–E correspond with impurities mentioned in the BP. The main attention is paid to the Bppa and the limit is the same as in the BP [31].

The critical point of Ibu assays, mentioned in the European Pharmacopoeia and in the BP, is the separation of the main peak and Bppa, because both individuals are chain isomers and their characteristics are very kindred [32], see Table 1. The chromatographic system can be used for Ibu evaluation only in the case that the ratio of peak height due to Bppa and the height above base-line of the lowest point of the curve separating this peak from the peak due to Ibu is greater than 1.5. If necessary the amount of acetonitrile in the mobile phase has to be adjusted in order to obtain the required resolution

Table 1
Comparison of physical–chemical characteristic of ibuprofen and 2-(4-*n*-butylphenyl)propionic acid (Bppa)

Ibu			Bppa		
Property	Value	Condition	Property	Value	Condition
H donors	1		H donors	1	
H acceptors	2		H acceptors	2	
Molecular weight	206.28		Molecular weight	206.28	
log *P*	3.722 ± 0.227		log *P*	3.906 ± 0.222	
log *D*	3.72	pH 1	log *D*	3.91	pH 1
log *D*	3.58	pH 4	log *D*	3.77	pH 4
log *D*	1.15	pH 7	log *D*	1.35	pH 7
log *D*	0.25	pH 8	log *D*	0.45	pH 8
log *D*	−0.036	pH 10	log *D*	−0.18	pH 10
pK_a	4.41 ± 0.20	Most acidic	pK_a	4.43 ± 0.20	Most acidic
Molar solubility	Sparingly soluble	pH 1	Molar solubility	Sparingly soluble	pH 1
Molar solubility	Sparingly soluble	pH 4	Molar solubility	Sparingly soluble	pH 4
Molar solubility	Slightly soluble	pH 7	Molar solubility	Slightly soluble	pH 7
Molar solubility	Soluble	pH 8	Molar solubility	Soluble	pH 8
Molar solubility	Very soluble	pH 10	Molar solubility	Very soluble	pH 10

[30,31]. Several authors have already dealt with the problems of separation of Ibu and its impurities [19–21,25,26]. Neither HPLC methods using silica based C18 columns as stationary phase [19,21], nor MEKC method [25,26] gave the sufficient resolution between Ibu and Bppa. Recently, has been reported a CEC method for quality control of Ibu by Quaglia et al. The peaks of Ibu and Bppa are separated quite well but the analysis time is about 100 minutes [33].

The goal of our study was to find better chromatographic conditions for the separation of Ibu and its related compounds, especially for the separation of Ibu and its chain isomer Bppa. The resolution is controlled by the selectivity factor (α), plate number (N) and retention factor (k'), and among these parameters, the selectivity factor has the most significant effect on resolution [34]. As a result, tuning the selectivity by changing the eluent type, the stationary-phase type, the eluent composition and sometimes the temperature can optimise the resolution. In fact, eluent type and the stationary-phase type are two of the most effective variables for modulating the selectivity [3]. That is why the polystyrene-coated zirconia stationary phase was chosen as a potential key to solving the above-mentioned separation problem.

2. Experimental

2.1. Instruments

All chromatographic work was performed on a Shimadzu chromatography system equipped with system controller SCL–10A$_{VP}$, detector SPD-10A$_{VP}$, pump LC-10AD$_{VP}$, autoinjector SIL-10AD$_{VP}$, column oven CTO-10AS$_{VP}$, degasser DGU-14A, low pressure module FCV-10AL$_{VP}$ and a computer-based chromatographic software Class-VP, ver. 6.12 Shimadzu (Tokyo, Japan). The UV–vis spectrometer UV2401PC Shimadzu (Tokyo, Japan) was used for measurement of the UV-spectra.

2.2. Chromatographic columns

In experimental work, following analytical HPLC columns were used: Discovery®Zr-PS, 150 mm × 4.6 mm i.d., particle size 5 μm, Sigma–Aldrich Chemie (Schnelldorf, Germany) and Sepharon SGX RPS, 150 mm × 4.0 mm i.d., particle size 5 μm, Tessek (Prague, Czech Republic).

2.3. Chemicals

Ibuprofen Sigma (St. Louis, MO, USA); 4-isobutylacetophenone 98% (Lancaster, UK); 2-(4-*n*-butylphenyl)propionic acid was purchased from Council of Europe, European Directorate for the Quality Control of Medicines (Strasbourg, France);

2-Hydroxy-2-(4-isobutylphenyl)propionic acid (2OH), 2-(4-isobutyrylphenyl)propionic acid (Bopa) and 2-(4-isobutylphenyl)propionamide (Amide), Zentiva, a.s. (Czech Republic). Methanol (MeOH), acetonitrile (ACN), tetrahydrofurane (THF), propane-2-ol (IPA), sodium dihydrophosphate p.a., phosphoric acid 85% p.a., sodium chloride p.a., sodium phosphate p.a. were obtained from Lach-Ner (Czech Republic); tetramethylammonium chloride was purchased from Fluka AG (Bushs SG, Switzerland).

2.4. Sample preparation

A mixture of methanol–water (50:50, v/v) was used as a solvent for preparation of all solutions. Stock solutions of Ibu impurities were prepared at concentration 1 mg ml^{-1}. The standard solutions were obtained by diluting the stock solutions to the concentration 0.006 mg ml^{-1} what corresponds to 0.3% of Ibu content (2 mg ml^{-1}). The test solution of Ibu was prepared by accurately weighing 20.0 mg of Ibu into 10 ml volumetric flask, dissolved and diluted to the mark. The solution with admissible amount of Ibu impurities (Limit solution) was prepared by accurately weighing 20.0 mg of Ibu into 10 ml volumetric flask and 60 μl from each stock solution of Ibu impurities was added and diluted to the mark.

2.5. Buffer preparation

- Phosphate buffer pH 6.4 was prepared by dissolving 2.5 g sodium phosphate, 2.5 g sodium dihydrophosphate and 8.2 g sodium chloride in 1000 ml flask [35].
- Phosphate buffer pH 2.33 – 50 mM phosphoric acid solution was adjusted with 1 M NaOH to pH 2.33.
- Phosphate buffer pH 4.5 – 50 mM sodium dihydrogenphosphate solution was adjusted with 5% phosphoric acid to pH 4.5.

3. Results and discussion

3.1. Alteration of eluent type

The problematic point of the pharmacopoeial assay [30,31] is to manage the sufficient resolution between peaks due to Ibu and Bppa. We focused on this problem firstly. As mentioned earlier the resolution can be optimised by tuning the selectivity by changing the eluent type, the eluent composition, temperature or the stationary phase. In the preliminary study MeOH instead of ACN in the mobile phase was tested to overcome the separation problem. Standard C18 column, as described in the Pharmacopoeias [30,31] was used. The best results were obtained with MeOH–phosphate buffer (pH 6.4) (45:55, v/v) containing 5 mM tetramethylammonium chloride as ion-pair additive, see Fig. 1. The results have shown, that there was a potential to achieve satisfactory separation with MeOH, but it was not possible to get better selectivity solely by altering the mobile phase composition.

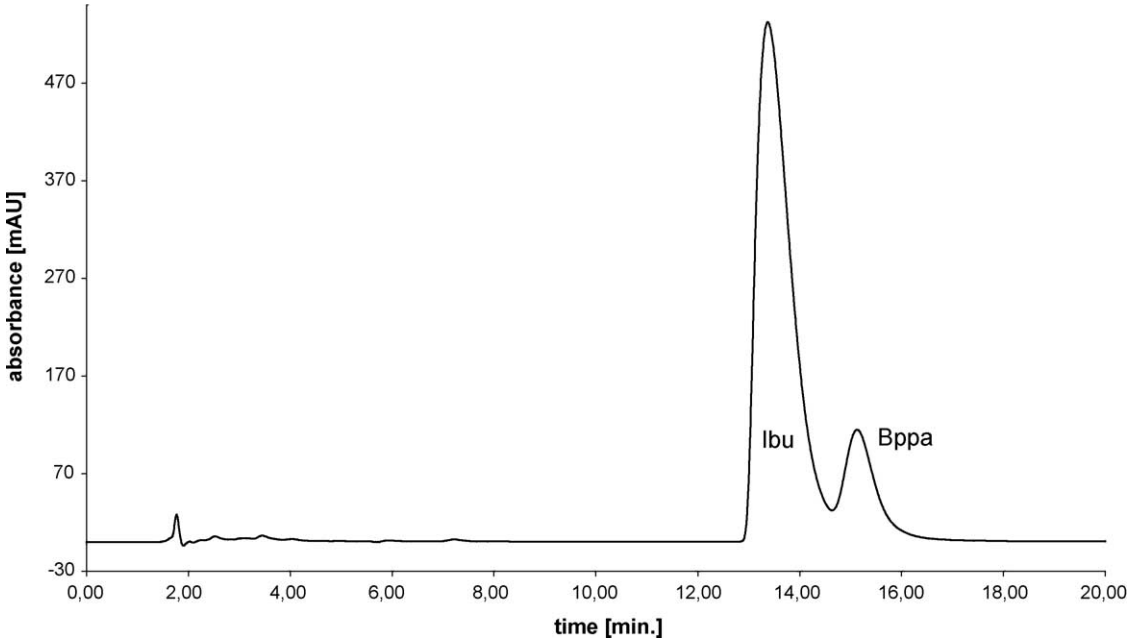

Fig. 1. Separation of Ibu and Bppa on silica based C18 column. Mobile phase MeOH–phosphate buffer (pH 6.4) (45:55, v/v) with 5 mM tetramethylammonium chloride; flow rate 0.5 ml min^{-1}, UV detection at 219 nm.

3.2. Alteration of stationary phase type

The stationary phase is another most effective variable for modulating the selectivity [3,34]. The retention mechanism on the zirconia-based stationary phases is a result of classical reversed-phase interactions and ion exchange properties. According to previously reported papers the PS-ZrO_2 column seemed to be good for our purpose – it shows good mass transfer characteristics, stability at extreme pH and temperature, substantial difference in selectivity compared to other phases, especially with respect to polybutadiene-coated zirconia [14,36].

We first tried to use the reverse phase mechanism that manifests especially at low pH area, for separation Ibu and Bppa. Lewis acid–base interactions on zirconia are characterised by especially slow desorption kinetics, which can broaden

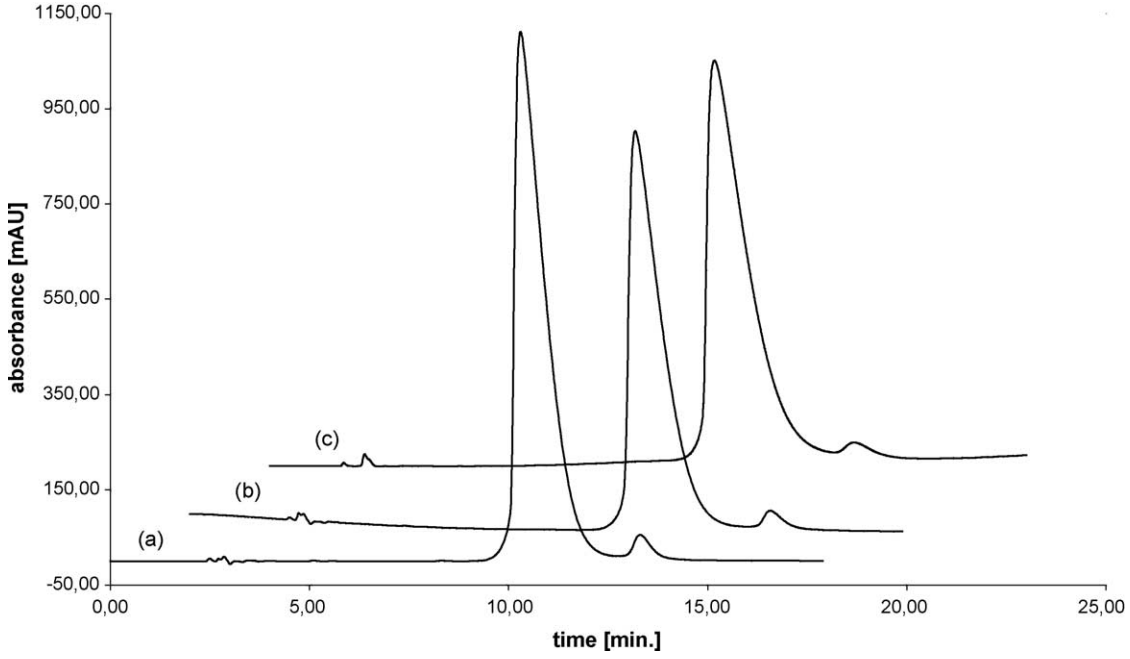

Fig. 2. Effect of different organic solvent on peak shape and symmetry on PS-ZrO_2 column. Mobile phase methanol–phosphate buffer (pH 2.33)–organic modifier (31:64:5, v/v/v). First peak Ibu, second peak Bppa; (a) tetrahydrofurane; (b) acetonitrile; (c) propane-2-ol.

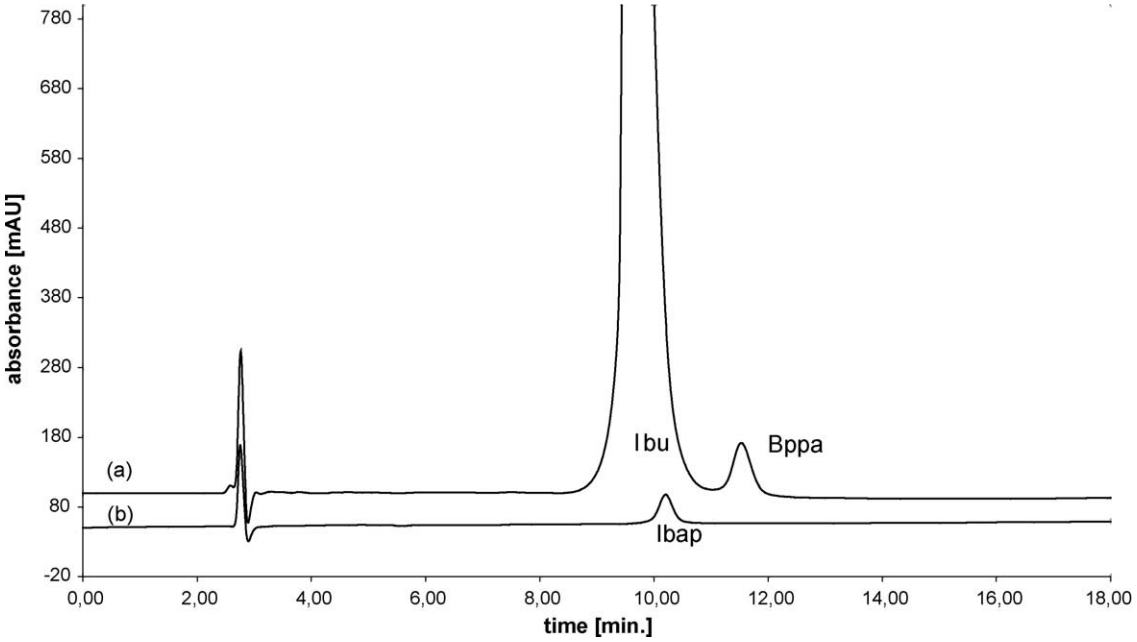

Fig. 3. (a) Separation of Ibu and Bppa on PS-ZrO_2 column. Mobile phase MeOH–phosphate buffer (pH 2.33)–tetrahydrofurane (30:65:5, v/v/v), flow rate 0.5 ml min^{-1}, column temperature 60 °C, UV detection at 219 nm. (b) Analysis of Ibap under the same conditions, detection at 258 nm.

peaks. However, addition of the hard Lewis base to the eluent (PO_4^{3-}, in our case) greatly improves the peak shape of the analytes. In order to improve more the peak profile a small amount of organic modifier was added and temperature was adjusted. IPA, ACN and THF were tested as possible organic modifiers. THF had the best effect on peak tailing, see Fig. 2. The temperature 60 °C proved to be sufficient for peak shape and resolution between the peaks. The best conditions were obtained with MeOH–phosphate buffer (pH 2.33)–THF (30:65:5, v/v/v), see Fig. 3a.

3.2.1. Zirconia-based stationary phase at optimal pH

The PS-ZrO_2 exhibits different selectivity toward silica-based stationary phases, and this fact manifested negatively on separation of degradation product Ibap from Ibu and Bppa. Ibap was co-eluted with the peak due to Ibu under pH 2.33,

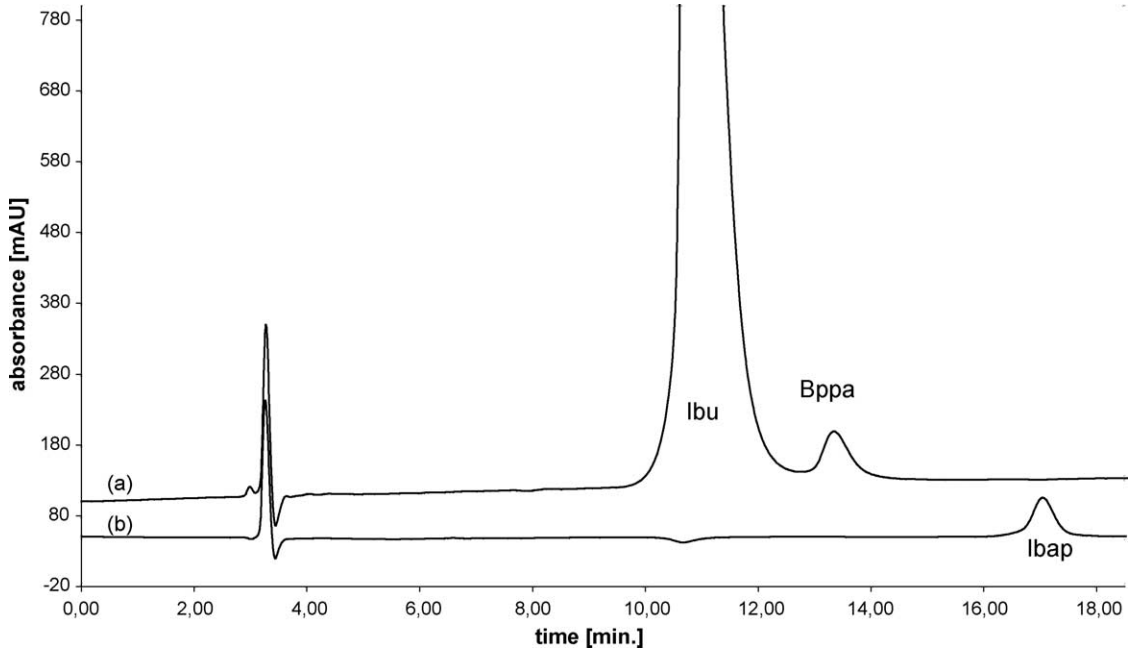

Fig. 4. (a) Separation of Ibu and Bppa on PS-ZrO_2 column. Mobile phase MeOH–phosphate buffer (pH 4.5)–tetrahydrofurane (25:70:5, v/v/v), flow rate 0.5 ml min^{-1}, column temperature 60 °C, UV detection at 219 nm. (b) Analysis of Ibap under the same conditions, detection at 258 nm.

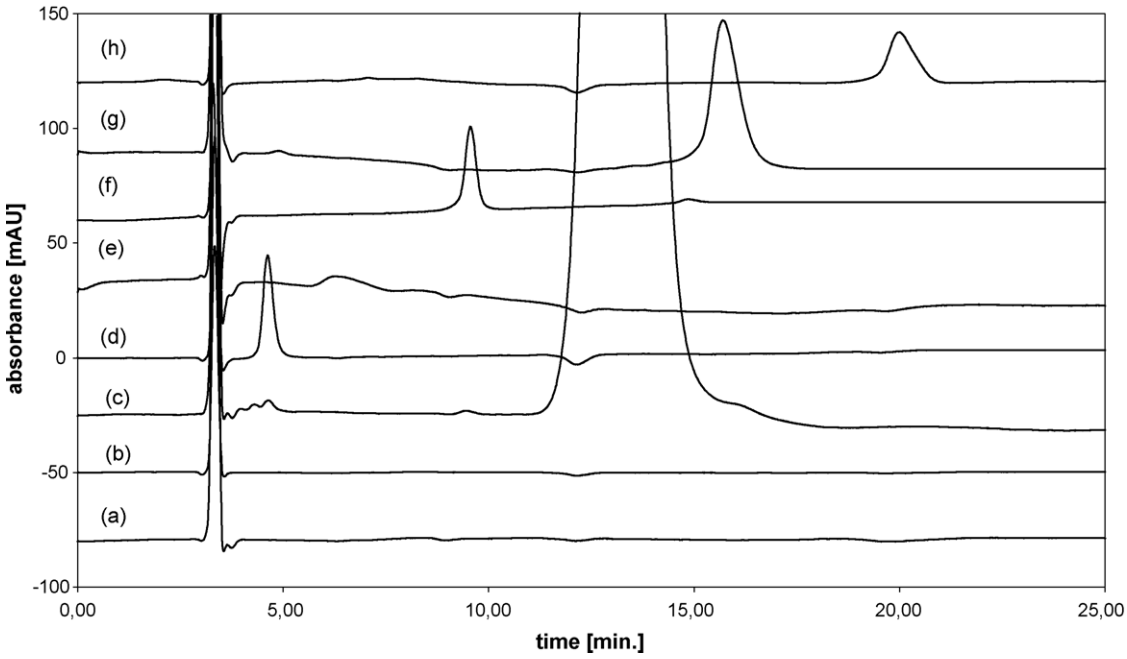

Fig. 5. Demonstration of the selectivity of the method. (a) Methanol–water (50:50, v/v), detection at 219 nm; (b) methanol–water (50:50, v/v), detection at 258 nm; (c) Ibu detection at 219 nm; (d) Bopa, detection at 258 nm; (e) 2OH, detection at 219 nm; (f) Amide, detection at 219 nm; (g) Bppa, detection at 219 nm; (h) Ibap, detection at 258 nm; All chromatograms were obtained under optimal conditions.

see Fig. 3. To overcome this problem the ion-exchange capability of the PS-ZrO_2 column was used. The mobile phases with different pH values were tested. The co-elution was still observed in the area of pH 2.33–4.0. The retention of Ibap was stronger in the range of pH values above 5.0 than that of Ibu and Bppa, but the separation of these two compounds got worse. The pH 4.5 was proved to be optimal for separation of all three compounds, see Fig. 4. If phosphate buffer was replaced with the acetate buffer at the same pH the analysis was impracticable. Acetate is a weaker Lewis base than phosphate is and the interactions between the analyte and stationary phase were so strong that unacceptable retention time (above 40 min) and peak tailing were occurred.

3.3. Separation of other related compounds

The pharmacopoeias [29–31] pay special attention to Bppa – impurity from the manufacture process [37], and Ibap – the main degradation product of Ibu [17]. The analytical profiles of drug substances describe 2-(4-isobutyrylphenyl)propionic acid (Bopa) as another possible impurity of Ibu, which was observed together with Ibap after exposition to harsh conditions such as 1 M NaOH, 1 M HCl or 50% H_2O_2. Ibap arises via radical induced decarboxylation followed by benzylic oxidation [17]. In the recently published paper Bopa is presented as a product of oxidation process [38]. Hence presence of Ibap and/or Bopa is indicating a decomposition of Ibu. 2-(4-Isobutylphenyl)propionamide (Amide) and 2-hydroxy-2-(4-isobutylphenyl)propionic acid (2OH) described as impurities C and M, respectively [31] were separated successfully under these conditions too, see Fig. 5.

3.4. Choice of an optimal wavelength

The related compounds of Ibu (Bppa, Amide, 2OH) have the local absorption maxima around 220 nm. The degradation products (Ibap, Bopa) exhibit bathochromic shift and the maxima of absorption are shifted to 258 nm, see Fig. 6. Unfortunately in the area around 220 nm these compounds have local minima, so the sensitivity of detection is very low. In order to gain maximal sensitivity of the assay the dual-wavelength modus at 219 nm and 258 nm was performed.

3.5. Optimal chromatographic condition for analysis of Ibu impurities

Mobile phase MeOH–phosphate buffer (pH 4.5, 50 mM)–THF (21:74:5, v/v/v), detector in the dual-wavelength modus set at 219 nm and 258 nm, flow rate 0.5 ml min^{-1} and the column temperature 60 °C are the optimal chromatographic conditions for the analysis of all above-mentioned compounds. The analysis under these conditions is completed within 22 minutes. The applicability of the method was also tested.

3.5.1. Linearity

The linearity test was performed using five concentrations levels for each impurity i.e. Bopa, 2OH, Amide, Bppa and Ibap in the range 0.03–0.36% of Ibu content. The linearity of

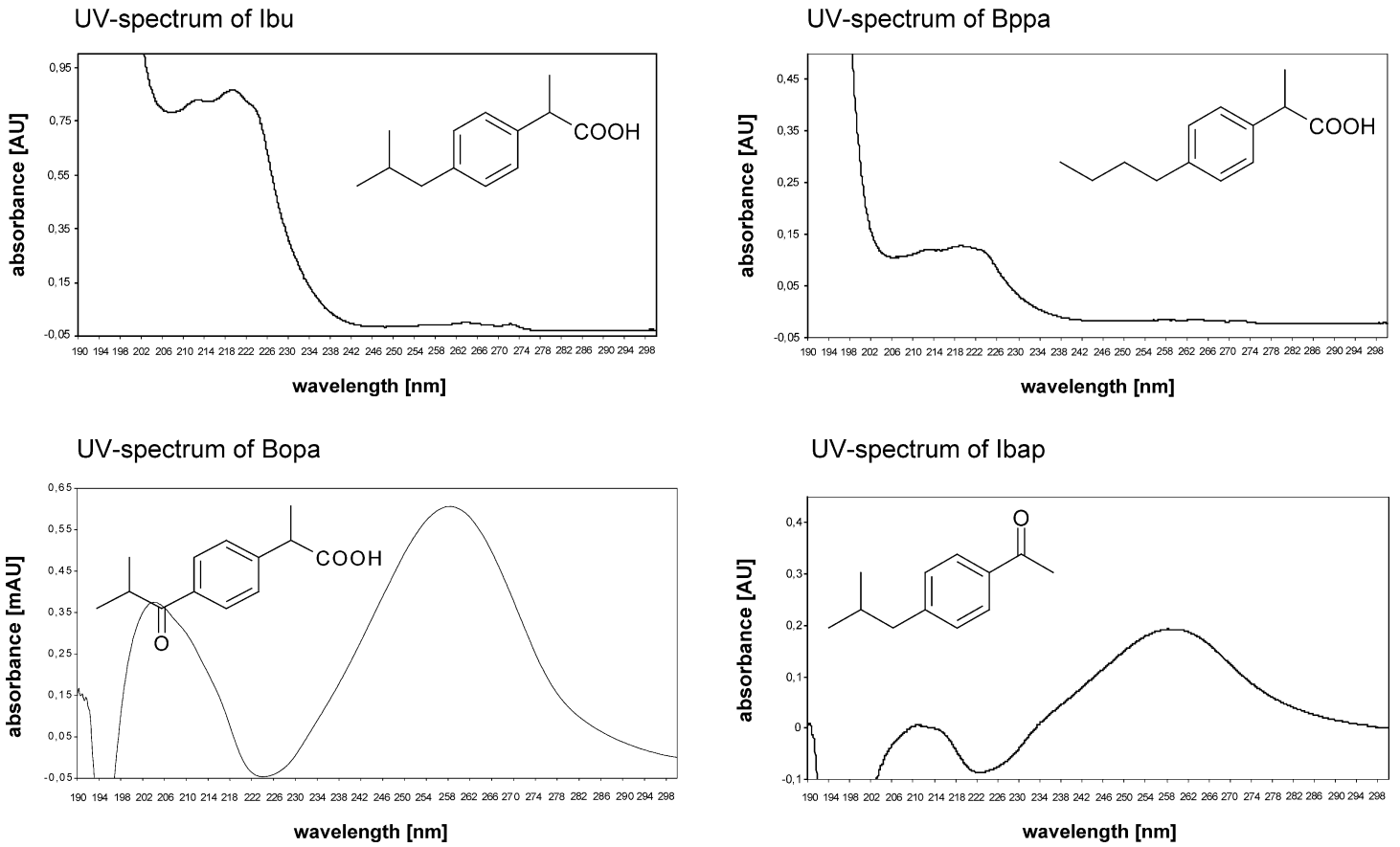

Fig. 6. Differences in the UV-spectra of Ibu, its related compounds and degradation products.

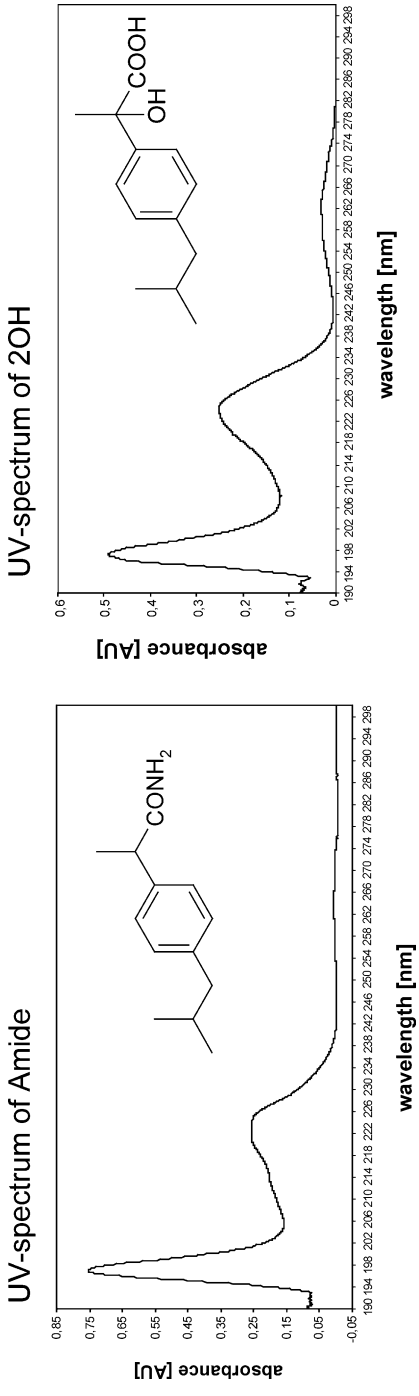

Fig. 6. (*Continued*).

Table 2
Limits of detection (LOD) and limits of quantification (LOQ) of each analyte

Analyte	LOD (mg ml^{-1})	LOQ (mg ml^{-1})
Bppa	4.56×10^{-5}	1.52×10^{-4}
Bopa	4.73×10^{-5}	1.57×10^{-4}
Ibap	5.22×10^{-5}	1.74×10^{-4}
Amide	3.47×10^{-4}	1.15×10^{-3}
2OH	8.22×10^{-4}	2.74×10^{-3}

Table 3
Response factors of each analyte

Analyte	Response factor
2OH	0.14
Amide	0.14
Bppa	2.78
Bopa	16.67
Ibap	33.33

Ibu was tested in the range 80–120%. The correlation coefficients, *r*, found were higher than 0.999.

3.5.2. Limit of detection (LOD) and limit of quantification (LOQ)

A signal-to-noise (S/N) ratio of 3 is generally considered to be acceptable for estimating the detection limit. A typical S/N ratio for calculating the quantitative limit is 10:1. Both limits were calculated, see Table 2.

3.5.3. Response factor

The responses of the detector to 0.3% solutions of 2OH, Amide and Bppa at 219 nm were divided by response of the detector to a 0.3% solution of Ibu at 219 nm. The response factors for Bopa and Ibap were calculated for wavelength 258 nm in the same way, see Table 3. The response factors differ considerably from each other; and thus it is better to prepare the solution with limit concentration of Ibu impurities for verification of Ibu quality, see Fig. 7.

3.5.4. Selectivity

The chromatograms obtained under optimal conditions, Fig. 5, exemplify the selectivity for Ibu, Amide, 2OH, Bppa, Bopa and Ibap. All the compounds were well separated within 22 minutes.

3.5.5. Robustness

The problematic point of the assay was to manage the sufficient resolution between peaks due to Ibu and Bppa and this criterion was the parameter for robustness test. The parameters, which might influence the resolution, (composition of mobile phase ±2% of MeOH, pH ±0.2, temperature ±5 °C, flow rate ±0.1 ml min^{-1}) were implied in the test. These small changes do not affect substantially the resolution between Ibu and Bppa.

Interesting results were obtained with the sample that was dissolved not in methanol but in acetonitrile. The peaks of Ibu and Bppa were not sufficiently resolved. The solvent has importance for achieving the expected selectivity of the assay. The conclusion is that the method is robust enough for evaluation of tested Ibu related compound and degradation products.

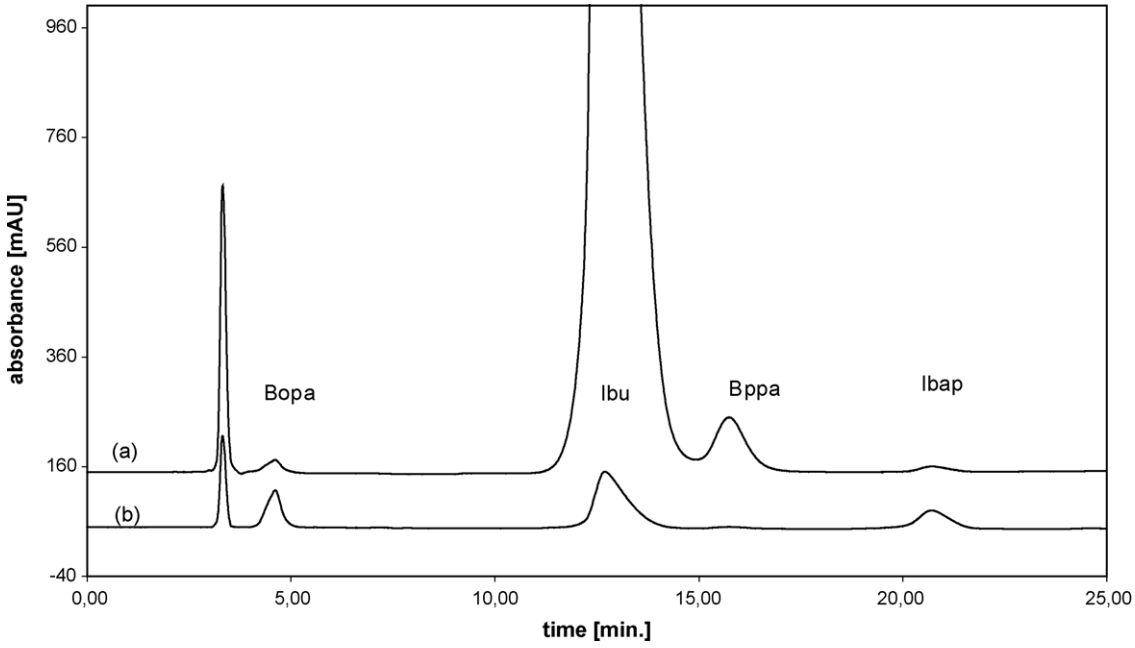

Fig. 7. (a) Separation of Ibu decomposition products (Bopa, Ibap) and Bppa under optimal conditions; detection performed at 219 nm. (b) Separation of Ibu decomposition products (Bopa, Ibap) and Bppa under optimal conditions; detection performed at 258 nm.

4. Conclusions

Zirconia-based stationary phase coated with polystyrene is substantially different from the conventional C18-SiO_2. We used this column with advantage for a HPLC separation of ibuprofen from some related compounds and its two decomposition products. This type of column offers both wide range of pH and also wide range of temperature to achieve optimal separation. It has been shown one practical example of employing zirconia-based stationary phase. Even though the carbon content of PS-ZrO_2 is much lower than that of conventional reversed-phase material, analytes exhibit sufficient retention and resolution, but with substantially improved analysis time. In conclusion, the method is applicable for evaluation of Bopa, 2OH, Amide, Bppa and Ibap in the raw material and for the monitoring of the degradation processes. It can be also used for the assay of ibuprofen. The method is simple, rapid and sensitive enough. The mobile phase contains less organic solvent than it is generally common. The flow rate is $0.5\,ml\,min^{-1}$, so the total amount of organic solvent consumption during analysis has been reduced to 18% of original value, which is not negligible from an environment viewpoint.

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