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Potential employment of non-silica-based stationary phases in pharmaceutical analysis

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Abstract

The absolute majority of the HPLC applications use silica-based columns for the separation of active substance and its impurities. However, stationary phases based on metal oxides appear as an interesting alternative. The aim of our study was to investigate the potential utilization of metal oxide-based stationary phases in analytical evaluation of ondansetron and its five pharmacopoeial impurities. In our study commercially available ZrO_2-based columns (e.g. Zr-PBD, Zr-PS, Zr-C18) and TiO_2-based column were used. The effect of an organic modifier (type and ratio), a buffer (type, pH and concentration) and the influence of temperature was investigated. The separation of ondansetron and its five pharmacopoeial impurities was successfully accomplished on a Zirchrom®-PBD column using a mobile phase consisting of acetonitrile-ammonium phosphate (25 mM, pH 7.0) (18:82, v/v). Detection was performed at 216 nm and the analysis was completed within 7.5 min. The paper proves metal oxide-based stationary phases as an alternative to classical silica-based stationary phases in pharmaceutical analysis.

Keywords: Metal oxide-based stationary phase; Zirconia-based stationary phase; RP-HPLC; Ondansetron; Impurities

1. Introduction

Quality control of raw materials and consecutive monitoring of potency and safety of pharmaceutical products constitute an important current subject of investigation in pharmaceutics. The impurities present in a drug or a drug formulation lower its quality and potency. The impurities are either residuals from the drug synthesis (e.g. by-products, residual solvents, etc.) or compound arising from decomposition reactions. Products of the decomposition process could be, on the one hand, ineffective (i.e. the drug quality is debased) but, on the other hand, toxic and thus for safety the quantity of drug is reduced.

The analytes are usually more complex than the model mixtures used in studies describing features of analytical columns. The model mixtures contain mostly easily separable components (i.e. the same nature but different structure), whereas real samples involve both moieties with different as well as very similar properties. The resolution between peaks is a basic parameter, which is usually optimised during method development process for an analysis of related compounds in a drug. In fact, eluent type and stationary phase type present effective variables for modulating the selectivity, and thus also for achieving sufficient separation on an HPLC column [1,2].

Properties of allied compounds and degradation products, which occur in drugs as impurities, are often very similar to the parent drug substance. This is the reason why new analytical separation methods, which show high selectivity for separation of complicated mixtures, should be developed. The HPLC is without doubts the most important analytical procedure in the field of drug analysis. The absolute majority of applications use silica-based columns, even though the use of silica support is limited by pH and temperature in relation to column stability [3–8].

To overcome these disadvantages stationary phases based on metal oxides – zirconia, aluminia and titania – have been developed [7,9]. Zirconia-based stationary phases have been the most investigated metal oxides stationary phases up to date [1,2,7,9–21]. High thermal and chemical stability over a wide range of temperature (up to 200 °C) and pH (1–14) are the most useful features of zirconia-based stationary phases [1,11,14,16,17,20,22]. The other advantage of zirconia-based columns is their different selectivity compared to silica-based

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Table 1
Commercially available RP ZrO_2-based stationary phases and their properties

Stationary phase	Surface of stationary phase	Advantages, analysed substances	Stability
Zr-PBD	Polybutadiene-modified zirconia	Intended to bases, amines (similar to silica-C18)	150 °C, pH 1–13
Zr-Carbon C18	Octadecyl-modified carbon-clad zirconia	Partitioning mechanism, shape selectivity	200 °C, pH 1–14
Zr-Carbon	Carbon-clad zirconia	Geometric isomers and diastereomers	150 °C, pH 1–14
Zr-PS	Polystyrene-modified zirconia	Very hydrophobic compounds, basic compounds and amines	150 °C, pH 1–13

columns. This enables their convenient utilisation also at mild conditions. The zirconia-based columns are also feasible for analysis of carboxylic compounds besides basic analytes. The potential problematic strong interactions with Lewis acids on the zirconia surface can be reduced due to addition of Lewis base (PO_4^{3-}, F^-, CH_3COO^-) into a mobile phase [7]. Nowadays four reversed-phase ZrO_2-based stationary phases are commercially available: Zr-PBD, Zr-Carbon C18, Zr-Carbon and Zr-PS. Each type of column possess different retention properties and has been developed for different analytical purpose [23,24] (see Table 1).

The next metal oxide studied as a chromatographic support is aluminia. Al_2O_3-based packings cannot be used for analytes containing carboxylic groups, because these compounds are bonded irreversibly on the aluminia surface. Moreover, except for mobile phases with pH above 10, aluminia-based packings have separation properties quite similar to those for silica, without obvious advantages [5].

The other metal oxide, which can be used as stationary phase bed for HPLC is TiO_2. Its properties and usefulness for chromatography have been investigated recently. There is still lack of data describing its properties and applicability to separation purposes. Some stationary phases based on TiO_2 have been used to separate basic or non-basic molecules under normal phase conditions [25,26]. Polyethylene-covered TiO_2 stationary phase has been developed for reversed-phase separation. The polymer layer causes decrease of Lewis acid–base interactions and thus improves the peak shape [7]. This stationary phase should have similar properties as Zr-PBD [23,27], which enables its utilisation as an alternative, when separation on Zr-PBD is insufficient.

The serotonin (5-hydroxytryptamine; 5-HT) type 3 receptor ($5\text{-}HT_3$) antagonist ondansetron has become first line therapy for the treatment of postoperative nausea and emesis as well as of emetogenic side effects accompanying cancer chemotherapy. Its quality assurance is implicit just as the other pharmacopoeial substances and pharmacologically active compounds present in pharmaceutical preparations of current use.

Racemic ondansetron hydrochloride dihydrate has been firstly mentioned in the 5th edition of European Pharmacopoeia (Ph. Eur.) [28], whereas the United States Pharmacopoeia, has mentioned ondansetron 6 years ago in the 24th edition [29]. The Pharmacopoeias [28,30,31] use nitrile silica-based stationary phase for determination of ondansetron and its impurities (except of impurity B) and the retention time of the last substance (ondansetron) is about 18 min.

Silica-based stationary phases are mostly used for HPLC analysis of ondansetron in human plasma [32–35] and pharmaceutical preparations [36–39] according to the literature. No publication has described a method for determination of ondansetron and its impurities and degradation products.

The aim of our work was to investigate the potential utilization of non-silica-based stationary phases in analytical evaluation of ondansetron and its five pharmacopoeial impurities (Fig. 1). Commercially available ZrO_2-based columns (DiamondBond®-C18, ZirChrom®-PBD, Discovery®Zr-PS) and TiO_2-based column (Sachtopore®-RP) were used in our study. The retention behaviour of analysed substances has been investigated.

2. Experimental

2.1. Instruments

All chromatographic experiments were performed on a Shimadzu chromatography system assembled of communication bus module CBM 20A, DAD detector SPD-M20A, pump LC-20AD, autoinjector SIL-20AC, column oven CTO-20AC, degasser DGU-$20A_3$ and a computer-based chromatographic software LC solution, Shimadzu (Tokyo, Japan).

2.2. Chromatographic columns

The following analytical columns were used during experiments: Zorbax SB-Aq, 150 mm × 4.6 mm i.d., particle size 3.5 μm, HPST (Czech Republic), DiamondBond®-C18, 150 mm × 4.6 mm i.d., particle size 5 μm, Sachtopore®-RP, 150 mm × 4.6 mm i.d., particle size 5 μm, ZirChrom®-PBD, 150 mm × 4.6 mm i.d., particle size 5 μm, ZirChrom Separations (Anoka, USA), Discovery®Zr-PS, 150 mm × 4.6 mm i.d., particle size 5 μm, Supelco-CN, 120 mm × 4.6 mm i.d., particle size 5 μm, Sigma–Aldrich (Schnelldorf, Germany).

2.3. Chemicals

Ondansetron hydrochloride dehydrate – (3*RS*)-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one – and its five pharmacopoeial impurities (Fig. 1) were kindly provided by Zentiva a.s. The other chemical substances came from common commercial sources.

2.4. Standard solutions

- Stock solutions of ondansetron and its five impurities were prepared at concentration 1 mg mL^{-1} in methanol.

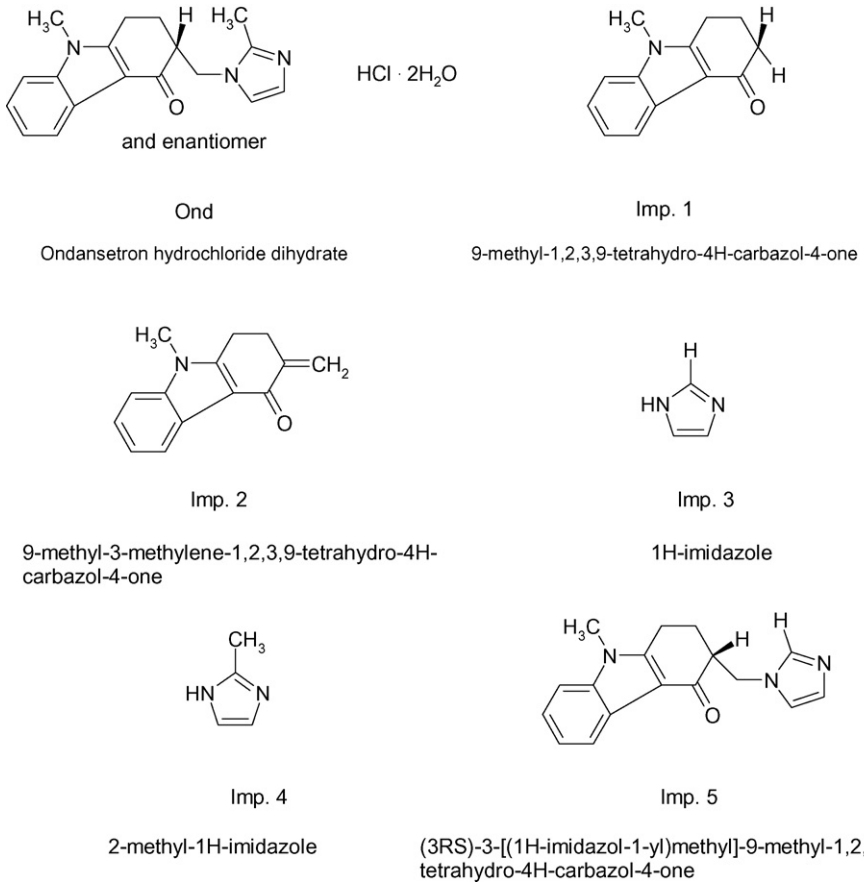

Fig. 1. Chemical structures of analysed compounds.

- Standard solutions were prepared by diluting stock solutions with methanol to the concentration 20 μg mL^{-1}.
- The same operation was used for preparation of mixture of ondansetron and its five impurities.

For determination of dead volume, potassium iodide (1 mg mL^{-1}) for silica-based columns and acetone (1 mg mL^{-1}) for zirconia-based and titania-based columns, were used. The injection volume of standard solutions was 5 μL.

2.5. Model sample

The model sample of ondansetron hydrochloride dihydrate, containing admissible amount of impurities, was prepared as follows: the stock solutions was diluted by mobile phase to these final concentrations: Imp. 1, 3, 4 and 5—1 μg mL^{-1}; Imp. 2—0.5 μg mL^{-1}; Ond—500 μg mL^{-1} [28]. The injection volume of model of impure ondansetron was 20 μL.

2.6. Buffer preparation

Buffers were prepared by dissolving appropriate salt (ammonium dihydrogenphosphate, diammonium hydrogenphosphate and ammonium fluoride or ammonium acetate) in water, and the pH value was adjusted by addition of 0.1 M ammonium hydroxide or 10% phosphoric acid.

3. Results and discussion

The separation of ondansetron and its impurities was tested on six HPLC columns. A special attention was paid to the influence of the experimental conditions on separation of studied compounds on metal-based stationary phases. The effect of an organic modifier (type and ratio), a buffer (type, pH and concentration) and the influence of temperature was investigated. All performances were realised using universal detection wavelength 216 nm and the ratio of acetonitrile in a mobile phase was 20% [28].

Achieving the sufficient resolution between imidazole (Imp. 3) and 2-methylimidazole (Imp. 4) constituted the main problematic point of the separation on all stationary phases. The experiments and obtained results are summarised for each column below.

3.1. Silica-CN

Using 20% acetonitrile in the mobile phase resulted in weak retention of all analysed substances on silica-CN stationary phase ($k'_{\mathrm{Ond}} = 1.33; k'_{\mathrm{Imp.\,3,4}} = 0$). The effect of pH and buffer concentration on retention of first eluted compounds – Imp. 3 and 4 – was negligible. These compounds were eluted constantly in dead retention time. The only way how to attain sufficient retention of both impurities was decreasing the amount of the organic modifier. Even if the mobile phase was composed of

acetonitrile-ammonium phosphate (pH 5.4; 20 mM) (5:95, v/v) the retention factor of Imp. 3 and 4 was insufficient and both impurities were eluted at the same retention time as potassium iodide. Further decreasing of the amount of acetonitrile in the mobile phase was improper with respect to stability of silica-based stationary phase [5].

The studied silica-CN stationary phase appeared unsuitable for the separation of ondansetron and its five impurities.

3.2. Silica C18

Zorbax SB-Aq is an alkyl reversed-phase bonded phase especially designed to operate under highly aqueous condition (including 100% water phase) for sufficient retention of hydrophilic compounds [40].

Retention of analysed substances was naturally higher in comparison with silica-CN column, but unfortunately the selectivity for above mentioned Imp. 3 and 4 was also insufficient. The effect of a pH value and a buffer concentration on retention and selectivity of these analytes, i.e. Imp. 3 and 4, was minimal. The ratio of acetonitrile in mobile phase affected strongly the retention of all analysed compounds. One-minute increase in retention time of Imp. 4 was followed by 40 min rise in retention time of last eluted substance—Ond (flow rate was 1.50 mL min^{-1}). The effect of pH and concentration of a buffer on retention of ondansetron was negligible. Higher resolution than 2.00, between peaks due to Imp. 3 and 4 could be achieved, but the total analyses time for isocratic elution would be higher than 60 min. That is why this column appeared improper for isocratic separation of ondansetron and its five impurities.

3.3. Zr-PS

Zr-PS stationary phase is made of ZrO_2, which is covered by a polystyrene layer [24]. This column has been developed for separation of very hydrophobic compounds, basic compounds and amines.

The order of eluted substances on Zr-PS stationary phase remained the same as on silica-based columns. Using mobile phase composed of acetonitrile-ammonium phosphate (pH 5.4; 20 mM) (20:80, v/v) Imp. 3 was eluted at the same retention time as dead retention time indicator—acetone. There was practically no observable resolution between peaks of first eluted substances. The effect of pH and buffer concentration on retention of Imp. 3 and 4 was minimal with 20% acetonitrile in mobile phase. Decrease of amount of acetonitrile in mobile phase was essential to increase retention of Imp. 3 and 4 and to examine the effect of buffer pH and buffer concentration on resolution between bands of Imp. 3 and 4.

The best results were obtained by using mobile phase composed of acetonitrile-diammonium phosphate (pH 7.5; 20 mM) (7:93, v/v), column temperature 50 °C and flow rate 1.50 mL min^{-1}. After all, the resolution between peaks due to Imp. 3 and 4 was 1.20 and the resolution between Imp. 2 and 5 (fourth and fifth eluted compound) was 1.45. The last two peaks (Imp. 5 and Ond) were broad and tailed and total analysis time was about 32 min.

Due to inappropriate peak shapes of Imp. 5 and Ond and also relatively long time of analysis, Zr-PS column appeared improper for isocratic separation of ondansetron and its five impurities.

3.4. Zr-C18

DiamondBond®-C18 stationary phase belongs also to a group of zirconia-based columns. The stationary phase is analogous to conventional ODS columns. The C18 ligands are attached to the carbon layer on the surface of zirconia with ultra-stable carbon–carbon bonds—so the columns are impervious to extremes mobile phase chemistry and temperature [23]. This stationary phase is ideal for the reversed-phase separation of positional isomers and diastereomers [23,24].

The retention of analysed compounds on Zr-C18 column was obviously the highest of all investigated columns as was expected. Double amount of acetonitrile in mobile phase, compared to the other investigated columns, was essential for achieving separation of ondansetron and its studied impurities in acceptable time. The best results were obtained by using mobile phase composed of acetonitrile-diammonium phosphate (pH 8.0; 20 mM) (50:50, v/v), column temperature 50 °C and flow rate 1.50 mL min^{-1}. The separation of all compounds was achieved. The retention factor of first eluted substance (Imp. 3) was only 0.16 but the retention factor of the last eluted substance (Ond) was 88.41 and tailing factor of Ond was 3.2.

Due to inappropriate peak shape of ondansetron and high total time of analysis, DiamondBond®-C18 column appeared improper for isocratic separation of ondansetron and its five impurities.

3.5. Titania-RP

Sachtopore®-RP stationary phase is a new type of metal-based RP stationary phase. It is made of TiO_2 and covered by a thin layer of polyethylene [23].

The similar selectivity towards Imp. 3 and 4 has been observed also on this column. After preliminary experiments the best results were achieved with acetonitrile-ammonium phosphate (pH 6.0; 20 mM) (18:82, v/v) as a mobile phase, column temperature 50 °C and flow rate 1.50 mL min^{-1}. Under these conditions the first eluted substance was Imp. 4 ($k' = 0.58$) and then Imp. 3 as opposed to other columns. The resolution between them was 2.13. The tailing factor of peak of the last eluted compound (Ond) was 1.41. The total analysis time was reduced to 10.5 min.

The Sachtopore®-RP column appeared suitable for isocratic separation of ondansetron and its five impurities. Since titania-RP stationary phase is similar to Zr-PBD stationary phase [23] also the Zr-PBD column has been tested before detailed study of retention behaviour was performed.

3.6. Zr-PBD

Zr-PBD stationary phase is made of ZrO_2 and its surface is covered by polybutadiene layer.

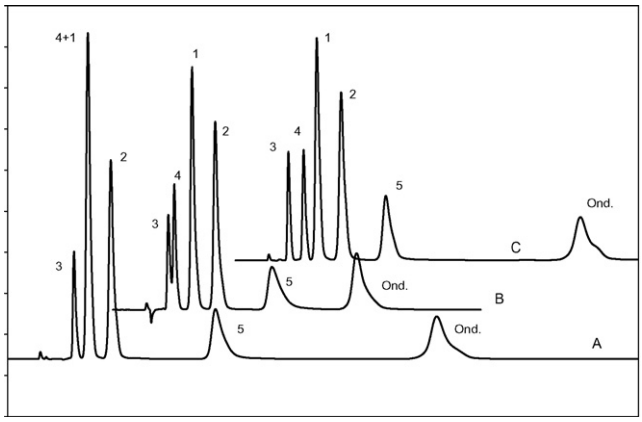

Fig. 2. Influence of Lewis-base additives on column selectivity; conditions: temperature 50 °C, flow rate 1.50 mL min^{-1} and mixture of acetonitrile-buffer (20 mM, pH 7.0) (18:82, v/v) as mobile phase; A, ammonium fluoride; B, ammonium acetate; C, ammonium phosphate.

Satisfactory separation on Zr-PBD under the same conditions as on titania-based column was not achieved. There was practically no resolution among peaks of Imp. 2–4. Increase of pH to 7.0 was sufficient for satisfactory separation of all analysed compounds in total analysis time 9.5 min. In contrast to titania-RP column the first eluted substance on Zr-PBD column was Imp. 3.

Owing to sufficient separation of all analysed compounds in total analyses time 9.5 min, Zr-PBD column was chosen for further experiments. The aim was to describe the influence of experimental conditions on retention behaviour of studied analytes and optimization of separation.

3.6.1. Effect of type, pH and concentration of buffer

Ammonium phosphate, ammonium fluoride and ammonium acetate were tested as Lewis-base additives to the mobile phase. The presence of Lewis-base in the mobile phase influences the interaction between an analyte and Lewis-acid sites on the surface of zirconia.

The fluoride anions as a constituent of mobile phase affected the selectivity of the stationary phase, and thus coelution of peaks due to Imp. 4 and 1 was observed. Simultaneously the retention of ondansetron slightly increased comparing to the mobile phase with phosphate instead of fluoride.

When fluoride is replaced by acetate the cation-exchange interactions are weaker. Therefore, the retention of all compounds is lower. The resolution between peaks of Imp. 3 and 4 was improved, in contrast to mobile phase with fluoride. The best result were obtained using phosphate buffer as a constituent of mobile phase (see Fig. 2).

The influence of buffer pH on separation of all six analysed compounds was investigated in pH range of 2.0–9.0. The dependence between retention factor k' and pH of buffer using mobile phase acetonitrile-ammonium phosphate (20 mM) (18:82, v/v) is shown in Fig. 3. In respect of absence of the imidazole part of molecule with acid–base character the effect of pH of mobile phase on retention of Imp. 1 and 2 is negligible. The other investigated compounds showed the highest retention in the range of pH 4.5–5.0 and manifested retention behaviour typical for Lewis bases. Based on these results the pH value 7.0 was chosen. The time of analysis and robustness of the method, especially the resolution between two first peaks (i.e. Imp. 3, Imp. 4, respectively), was also taken into account.

The effect of buffer concentration was investigated in concentration range of 5–50 mM. The dependence between retention factor k' and buffer concentration is shown in Fig. 4. With regard to the neutral character of Imp. 1 and 2, no effect of buffer concentration on retention of these two compounds was observed. The retention of all other analysed substances and resolution due to peaks of Imp. 3 and 4 decreased with higher buffer concentration. The appropriate concentration of phosphate buffer to keep the sufficient resolution within the group of analytes was 25 mM.

3.6.2. Effect of organic modifier

The effect of acetonitrile (ACN) and methanol (MeOH) on retention of ondansetron and its impurities was examined. Relatively great difference between these organic modifiers was observed. MeOH in mobile phase caused long retention times, broader peaks and higher column pressure. For these reasons ACN was chosen as the organic modifier.

The influence of ratio of acetonitrile in the mobile phase on separation was studied in range 14–22% (v/v) (5 values) (flow rate 1.50 mL min^{-1}, 50 °C, 25 mM ammonium phosphate buffer, pH 7.0). The reversed-phase behaviour was observed, i.e. an increase of acetonitrile in mobile phase caused decrease

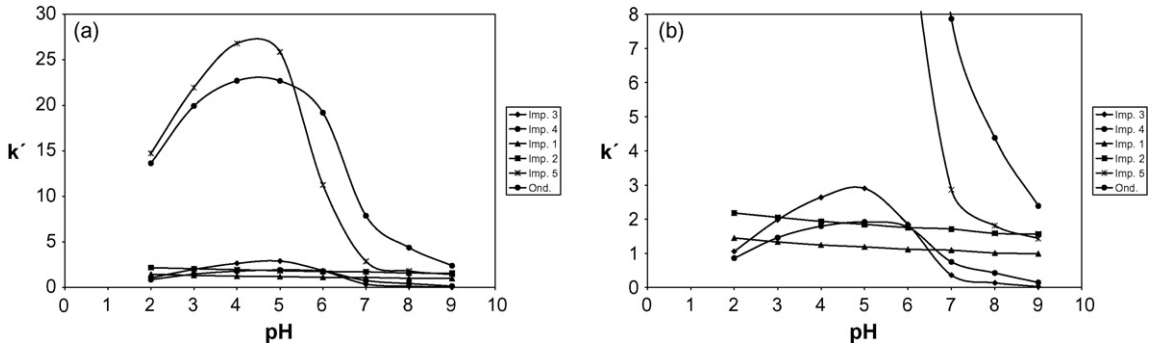

Fig. 3. The dependence between retention factor k' and pH of buffer; conditions: temperature 50 °C, flow rate 1.50 mL min^{-1} and mixture of acetonitrile-ammonium phosphate (20 mM) (18:82, v/v) as mobile phase (a) and detailed image (b).

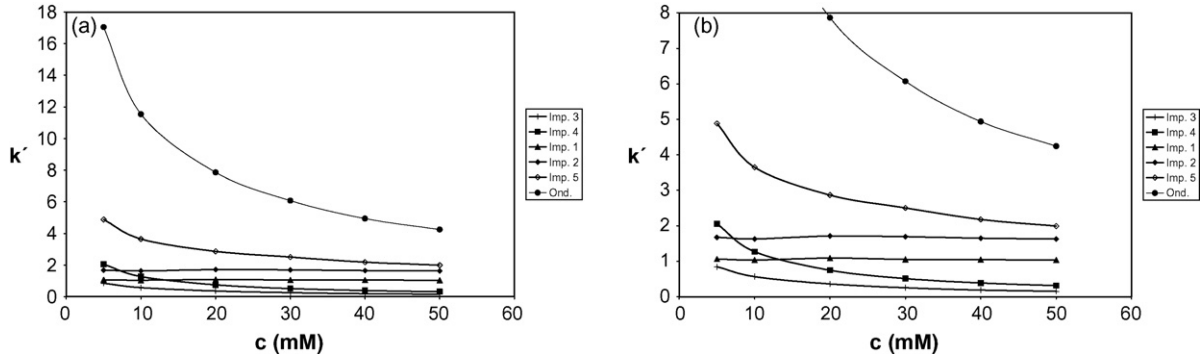

Fig. 4. The dependence between retention factor k' and concentration of buffer; conditions: temperature 50 °C, flow rate 1.50 mL min^{-1} and acetonitrile-ammonium phosphate (pH 7.0) (18:82, v/v) as mobile phase (a) and detailed image (b).

of retention of all analysed compounds. Higher amount of acetonitrile in mobile phase than 20% (v/v) resulted in resolution between peaks of Imp. 1 and 4 under the value 2.00. According to these facts and sufficient method robustness, the best ratio for separation of ondansetron and its five impurities appeared 18% (v/v).

3.6.3. Effect of temperature and flow rate

The effect of column temperature on separation was investigated in the range 40–70 °C (4 values) under following condition acetonitrile-ammonium phosphate (25 mM, pH 7.0) (18:82, v/v), flow rate 1.50 mL min^{-1}, Increase in column temperature resulted in better peak shape, lower column pressure, gentle decreasing of retention of all analysed compounds and implicitly in decrease of total analysis time. But the resolution between peaks of Imp. 3 and 4 and between Imp. 2 and 5 decreased. Setting up the column temperature higher than 60 °C, the resolution between peaks of Imp. 2 and 5 decreased below 2.00. Column temperature higher than 70 °C induced decrease of resolution between peaks of Imp. 3 and 4 under the value 1.95. In view of sufficient method robustness and potential analyte instability, the column temperature 50 °C was chosen as the best for separation of analysed compounds.

The influence of flow rate on separation was investigated in the range 1.25–3.50 mL min^{-1} (10 values), under following condition acetonitrile-ammonium phosphate (25 mM, pH 7.00) (18:82, v/v), temperature 50 °C. Flow rate above 3.00 mL min^{-1} reduced the resolution between peaks of Imp. 3 and 4 below the value 2.00. The flow rate 2.70 mL min^{-1} was chosen with respect to the analysis time and the method robustness.

3.6.4. Linearity, LOD and LOQ, precision, robustness

The optimised conditions for separation of ondansetron and its five impurities are mobile phase acetonitrile-ammonium phosphate (25 mM, pH 7.0) (18:82, v/v), column temperature 50 °C and flow rate 2.70 mL min^{-1}. All analysed compounds were separated with higher resolution than 2.00 under these conditions. The total analysis time was 7.5 min and column pressure was 15.1 MPa. The chromatogram of model sample, containing limit concentrations of Imp. 1–5 in presence of ondansetron [28] is presented in Fig. 5.

The suitability of the developed method was confirmed by means of linearity, precision, robustness and determination of LOD as well as LOQ.

Linear response of impurities was tested in the range 10–120% of the admissible amount (seven concentrations). Response of ondansetron is linear in the range 9.0–108.0 μg mL^{-1} (seven concentrations) [28]. The correlation coefficients (r) were higher than 0.997 for all compounds (see Table 2).

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).

Precision was determined using spiked standard substance of ondansetron (six preparations). Relative standard deviation values (R.S.D.) were calculated for multiple sample preparations (method precision) (see Table 2).

The effect of small changes of analytical parameters on separation, was also investigated. The results have proven that pH of mobile phase in the range of 6.8–7.2 and buffer concentration in the range of 20–30 mM do not affect the separation process, however total analysis time is slightly affected. The column temperature should not be higher than 60 °C and flow rate not

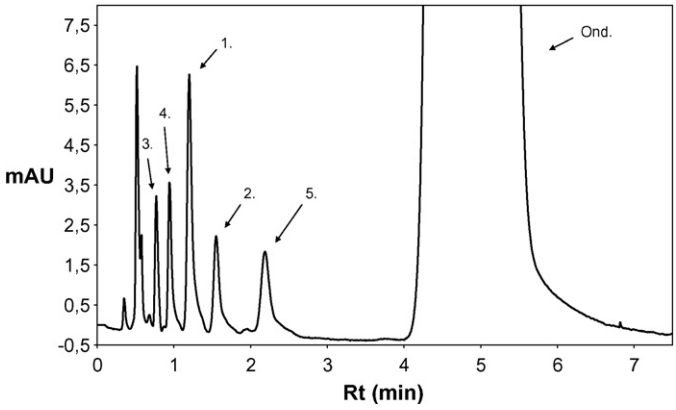

Fig. 5. The chromatogram of real pharmaceutical (ondansetron) spiked with admissible amount of studied impurities under the optimal conditions for separation; conditions: ZirChrom®-PBD column, acetonitrile-ammonium phosphate (25 mM, pH 7.0) (18:82, v/v) as the mobile phase, temperature 50 °C and flow rate 2.70 mL min^{-1}.

Table 2
Results of validated parameters for each analysed compound; Zr-PBD column, acetonitrile-ammonium phosphate (25 mM, pH 7.0) (18:82, v/v) as the mobile phase, 50 °C and flow rate 2.70 mL min^{-1}

	Imp. 1	Imp. 2	Imp. 3	Imp. 4	Imp. 5	Ond
Method precision						
R.S.D. [%]	2.11	2.12	2.45	1.54	2.34	2.41
Linearity						
Correlation coefficient	0.9984	0.9971	0.9984	0.9992	0.9978	0.9990
Slope	52.3	56.5	18.1	19.9	36.5	5.48
Intercept	−1.89	−2.84	−0.19	0.26	−4.14	−4.90
LOD						
μg mL^{-1}	1.5×10^{-2}	7.5×10^{-3}	1.5×10^{-2}	6.0×10^{-3}	1.5×10^{-2}	–
%	3.0×10^{-3}	1.5×10^{-3}	3.0×10^{-3}	1.2×10^{-3}	3.0×10^{-3}	–
LOQ						
μg mL^{-1}	5.0×10^{-2}	2.5×10^{-2}	5.0×10^{-2}	2.0×10^{-2}	5.0×10^{-2}	–
%	1.0×10^{-2}	5.0×10^{-3}	1.0×10^{-2}	4.0×10^{-3}	1.0×10^{-2}	–

Table 3
Response factors and correction response factors for each compound

Impurity	Response factor	Correction response factor
Imp. 1	0.44	2.27
Imp. 2	0.52	1.92
Imp. 3	1.33	0.75
Imp. 4	1.29	0.78
Imp. 5	0.80	1.25

higher than 3.00 mL min^{-1}. The maximal amount of acetonitrile in mobile phase must not get over 20%. According to these facts we found this method robust enough for analytical evaluation of ondansetron and its five impurities.

The responses of the detector to 20 μg mL^{-1} solutions of Imp. 1–5 at 216 nm were divided by response of the detector to 20 μg mL^{-1} solution of Ond at 216 nm (see Table 3). The response factors differ considerably from one another; and thus it is necessary to prepare the solution with limited concentrations of all impurities for purity control or use the reciprocal values of response factor—correction response factors.

4. Conclusion

Four HPLC metal oxide-based columns (Sachtopore®-RP, ZirChrom®-PBD, DiamondBond®-C18, Discovery®Zr-PS) and two silica-based columns (Zorbax SB-Aq and Supelco-CN) were tested for utilization in analysis of ondansetron and its pharmacopoeial impurities (Fig. 1). Different properties of zirconia with regard to silica allowed better separation of analysed compounds. The Zr-PBD column appeared as the best one. The optimization of chromatographic condition consisted in achieving satisfactory resolution between imidazole and 2-methylimidazole with regard to reasonable analysis time. The best results achieved on each column are shown in Table 4.

Optimal conditions for separation of ondansetron and its five impurities on Zr-PBD column were: acetonitrile-ammonium phosphate (25 mM, pH 7.0) (18:82, v/v) as the mobile phase, column temperature 50 °C and flow rate 2.70 mL min^{-1}. Detection was performed at universal wavelength 216 nm. Under these conditions six analysed compounds were separated with higher resolution than 2.00 and total analysis time was 7.5 min.

Table 4
Resolution between peaks due to Imp. 3 and 4 on each investigated column (mobile phase composed of ACN + 20 mM ammonium phosphate buffer, 50 °C and flow rate 1.5 ml min^{-1})

Column	Conditions	Resolution between peaks due to Imp. 3 and 4
Silica-CN	5 % ACN, pH 5.4	0.21
Silica C18	0% ACN, pH 5.4	1.35
Zr-PS	7 % ACN, pH 7.5	1.20
Zr-C18	50 % ACN, pH 8.0	2.22
Titania-RP	18 % ACN, pH 6.0	2.13
Zr-PBD	18 % ACN, pH 7.0	2.15

The developed method appears suitable for the monitoring of ondansetron and its five pharmacopoeial impurities. The possible applicability of zirconia-based stationary phase in pharmaceutical analysis was documented on a practical example. Simultaneous activity of ion-exchange and hydrophobic (reversed) interactions on zirconia-based stationary phase provides a wide range for modulating column selectivity and thus attaining appropriate resolution between analytes in the mixture, unlike silica-based stationary phase.

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