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A comparison of performance of various analytical columns in pharmaceutical analysis: conventional C_{18} and high throughput C_{18} Zorbax columns

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Abstract

New improved types of analytical columns Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm) and Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm i.d., 1.8 μm) have been tested for determination of estradiol (active substance), methylparaben, propylparaben (preservatives) and estrone (degradation product) and compared with the conventional C_{18} columns (250 mm × 3.0 mm i.d., 5.0 μm). The Zorbax columns differ with their particle size, column length and ODS (octadecylsilica) type as well. Higher flow-rates (up to about 2.5 ml min^{-1}) could be applied regardless to back-pressure. The analysis – previously done at 40 °C – could be performed even at ambient temperature. Analytical run was shortened to 3.5 min (from 12 min used for the conventional C_{18} column) with the same or better retention characteristics. System suitability data for all Zorbax columns show the advantages of these columns for the practical use in routine quality control of pharmaceuticals, particularly from the point of view of speed of analysis and solvent consumption.
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Keywords: Estradiol; Zorbax column; HPLC

1. Introduction

Speed of analysis has become of a great importance in many application areas of HPLC like pharmaceutical, toxicological and clinical analysis, where it is important to increase throughput and reduce analysis costs. The simplest mode how to shorten analytical run is column length shortening. However, that could be risky, because complex mixture of compounds does not have to be separated well enough. Therefore it is necessary to increase column efficiency by reducing particle size, which means augmenting absorption surface of stationary phase. Thus the analysis could be performed during a shorter time with the same separation efficiency.

In our study analytical columns made by Agilent Technologies were tested and compared with conventionally used ODS analytical column. Agilent provides many types of analytical columns with regards for the purpose of analysis. Different types of ODS stationary phase modified according the range of pH can be used [1,2].

For the mid pH region 2–9 serves Zorbax Eclipse eXtra Densely Bonded (XDB) column. At this range of pH the silanols are more active and tailing interactions are more likely. To overcome this problem the columns are eXtra Densely Bonded and in addition double endcapped so as to cover as many active silanols as possible. The structure can be seen in Fig. 1. They are recommended to be convenient for neutral, basic and acidic compounds in this pH range. XDB columns are available with -C_{18}, -C_8 and -Phenyl stationary phase [1,2].

For the low range of pH 1–6 the most convenient are Zorbax StableBond analytical columns. They are made using patented, unique monofunctional silanes with bulky diisobutyl (stationary phase C_{18}) or diisopropyl (stationary phases -C_{18}, -C_3, -Phenyl, -CN, and -Aq) that sterically protect the siloxane bond. The structure can be seen in Fig. 2. For the improvement of peak shape, the high purity, low acidity silica instead of endcapping is used in order to provide better

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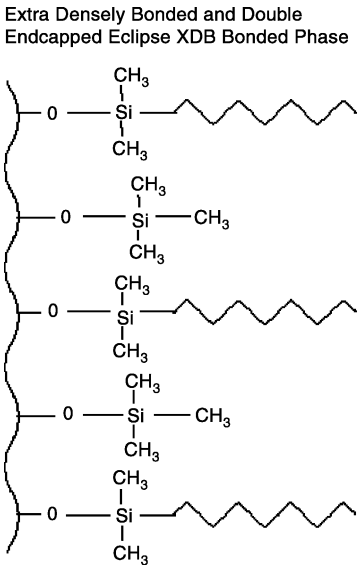

Fig. 1. The structure of Zorbax Eclipse eXtra Densely Bonded (XDB) stationary phase.

stability and to improve lifetime and reproducibility under acidic mobile phase conditions. SB columns are compatible with all common mobile phase, including very high aqueous mobile phases [1,2].

For the high pH range Zorbax Extend-C_{18} analytical columns should be used. They are made using new bidentate C_{18}–C_{18} column bonding technology. At the high pH, non-charged basic compounds do not interact with silica, so the separation is done with high efficiency as well the peak shape and resolution is improved. The Extend-C_{18} column is stable from pH 2–11 with a good peak shape for all types of compounds [1,2].

The above stated columns are available in all dimension from capillary to preparative columns, containing particles of usually 3.5 μm or 5.0 μm.

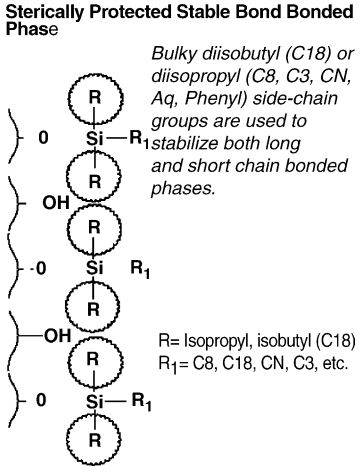

Fig. 2. The structure of Zorbax StableBond stationary phase.

Rapid resolution high throughput (HT) HPLC columns packed with 1.8 μm particles are a new family of LC and LC–MS columns. They are designed for very fast high-resolution separations. As the particle size of the column packing is decreased to 1.8 μm, column efficiency is increased. Therefore, these columns are very short (15–50 mm) and are intended especially for the fast separations applications. They are available with two bonded phases—Zorbax C_{18} Eclipse XDB and SB-C_{18}, which means compatibility with mobile phases from 1 to 9 pH range [3,4].

Just a few applications using 1.8 μm columns were described by the producer itself. They include analysis of some antibiotics (lincomycin and klindamycin) [3,5] and testing some standard sample mixtures containing acids, bases and neutrals as well [6]. The only application found in literature was describing the use 1.8 μm ODS modified particles in capillary chromatography [7]. Column frit preparation and column packing procedure, optimisation of mobile phase composition, acetonitrile content, surfactant concentration, pH, ionic strength as well as study of column efficiency was the objective of the paper.

Concerning 3.5 μm particles, much more practical use could be observed. The first use of packing with small particles (comparison of 3.5 μm particle packed column and traditional 5 μm particle column) was described in the early 1980s, but HPLC system compatibility and column lifetime issues did not allow the wide use of small particle packed columns [8]. In 1997 the comparison of 1.5 μm nonporous and 3.5 μm porous silica columns was made by Paasch et al. [9]. Determination of Ro 48-3656 as a prodrug considered to be an active antagonist of glycoprotein IIbIIIa in rat plasma was made. 3.5 μm particle packed columns were used especially in biological analysis, particularly in anticancer drug research [10,11]. Their use has increased during last four years with wider use of hyphenated techniques as liquid chromatography electro-spray ionization mass spectrometry (LC–ESI–MS) [12–15], liquid chromatography, tandem mass spectrometry (LC–MS–MS) [16,17], quadrupole time-of-flight spectrometry (Q-TOF MS) [18] and LC–UV–TOF [19] treating especially with analysis of drugs and their metabolites in biological fluids. Some papers describe the use of 3.5 μm particulate capillary columns in capillary chromatography [20] and electrochromatography [21].

Estradiol is successfully used in clinical practice for a treatment of climacteric syndrom symptoms and postmenopausal osteoporosis. Many different pharmaceutical formulations, especially tablets, transdermal plasters and topical gels, containing estradiol were developed for this purpose. Topical administration is very convenient, because estradiol in transdermal form is absorbed directly into the blood circulation, there is no influence of primary hepatic metabolism and fast degradation [22,23].

A novel analytical method for determination of estradiol (active substance), mehylparaben, propylparaben (preservatives) and estrone (degradation product) was recently developed in our laboratory [24].

The aim of this work was to compare a performance and retention data of different analytical columns containing octadecylsilica as a stationary phase using the method previously developed for pharmaceutical formulation Estrogel gel—examined substances are neutral compounds. Following analytical columns were tested: Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm) and Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm i.d., 1.8 μm**)**.

2. Experimental

2.1. Chemicals and reagents

Working standards of estradiol, estrone, methylparaben, propylparaben and hydrocortizone (internal standard) were used for the purpose of this study. The standards were provided by Sigma–Aldrich (Prague, Czech Republic). All these compounds were checked against European Pharmacopoeia CRS standards (Strasbourg, France).

Acetonitrile, Supragradient, was obtained from Biotech (Scherlau Chemie, Germany). HPLC grade methanol was provided by Sigma–Aldrich (Prague, Czech Republic).

HPLC grade water was prepared by Milli-Q reverse osmosis Millipore (Bedford, MA, USA) and it meets European Pharmacopoeia requirements.

2.2. Chromatography

Analyses were performed on Shimadzu LC-2010 C system (Kyoto, Shimadzu, Japan) with built-in UV–vis detector and with column oven enabling control of temperature. The built-in auto-sampler was conditioned at 25 °C. Chromatographic software Class VP 5 was used for data collection and processing.

The original chromatographic conditions for determination of Estrogel gel, (Discovery C_{18} (250 mm × 3.0 mm i.d., 5 μm), column oven temperature 40 °C, mobile phase acetonitrile, methanol, water (23:24:53) pumped isocratically at the flow-rate 0.9 ml min^{-1} detection at 225 nm injection volume 10 μl, using hydrocortizone as an internal standard (IS) for quantitation) were applied to Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm) and Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm i.d., 1.8 μm). Then the higher flow-rates were tested as follows: for the 3.5 μm particle sized column 2.5 ml min^{-1} was chosen as a maximum, for the 1.8 μm particle sized column 1.2 ml min^{-1} was chosen as a maximum flow-rate because of some system instabilities at higher flow-rates. System suitability data and repeatability data of all columns using different analytical conditions (40° a 25 °C, original and extreme conditions) were checked and compared.

2.3. Reference standard preparation

The stock solution of internal standard was prepared by dissolving 50 mg of hydrocortizone in 100 ml of ace-

Table 1
System suitability data

	Methylparaben				Hydrocortizone (IS)				Propylparaben				Estradiol				Estrone			
	t_r	N	H	R_{ii}	T	t_r	N	H	R_{ii}	T	t_r	N	H	R_{ii}	T	t_r	N	H	R_{ii}	T	t_r	N	H	R_{ii}	T
ODS 5 40°/0.9 ml min^{-1}	2.87	3077	6154	4.41	1.23	4.07	3013	6026	5.03	1.23	6.04	6000	12000	7.94	1.30	8.35	6381	12762	6.32	1.31	10.30	7756	15512	4.40	1.27
XDB 3.5 40°/0.9 ml min^{-1}	1.69	1918	25573	4.37	1.11	2.61	2514	33520	5.08	1.28	4.28	5969	79587	7.77	1.04	6.09	6741	89880	7.00	1.02	7.94	8207	109427	5.71	1.04
XDB 3.5 40°/2.5 ml min^{-1}	0.62	736	9813	2.78	1.29	0.97	1211	16147	3.44	1.28	1.54	2995	39933	5.14	1.11	2.23	3895	51933	4.83	1.06	2.89	4964	66187	4.33	1.02
XDB 3.5 25°/0.9 ml min^{-1}	1.93	2106	28080	6.75	1.05	3.07	2667	35560	5.63	1.28	5.40	6622	88293	9.28	1.10	7.97	7179	95720	8.01	1.03	10.76	8622	114960	6.63	1.10
XDB 3.5 25°/2.5 ml min^{-1}	0.70	1089	14520	2.37	1.19	1.14	1664	22187	4.38	1.28	1.95	3887	51827	6.79	1.05	2.93	4449	59320	6.55	1.10	3.92	5476	73013	5.10	1.03
XDB 1.8 40°/0.9 ml min^{-1}	1.23	1196	23920	1.99	1.19	1.94	1706	34120	4.26	1.28	3.05	4037	80740	5.88	1.09	4.47	5011	100220	6.37	1.01	5.71	6087	121740	4.56	1.05
XDB 1.8 40°/1.2 ml min^{-1}	0.93	1037	20740	3.62	1.13	1.46	1552	31040	4.07	1.20	2.29	3854	77080	4.23	1.13	3.37	4999	99980	5.71	1.10	4.31	6407	128140	3.76	1.07
XDB 1.8 25°/0.9 ml min^{-1}	1.39	1361	27220	1.93	1.19	2.25	1863	37260	4.77	1.28	3.80	4959	99180	7.30	1.04	3.80	4959	99180	7.30	1.04	7.59	7668	153360	5.75	1.05
XDB 1.8 25°/1.2 ml min^{-1}	1.05	1193	23860	1.90	1.07	1.70	1769	35380	4.63	1.28	2.84	4689	93780	6.96	1.15	4.34	6039	120780	7.67	1.15	5.70	7613	152260	5.65	1.13

t_r: retention time (min), N: theoretical plate number, H: height equivalent of theoretical plate (μm), R_{ii}: peak resolution, T: peak asymmetry, ODS 5: Discovery C_{18} (250 mm × 3.0 mm i.d.; 5 μm), XDB 3.5: Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm), XDB 1.8: Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm i.d., 1.8 μm), 40°/0.9: temperature 40 °C. Flow-rate 0.9 ml min^{-1}, 2.5 ml min^{-1}, 1.2 ml min^{-1}, respectively. *Six repetitions for each condition.

tonitrile. Reference standard solution for Estrogel analysis was prepared in 100 ml volumetric flask by dissolving of 1.5 mg of estradiol, 2.5 mg of methylparaben, 1.25 mg of propylparaben and 0.5 mg of estrone in acetonitrile. Finally 2.0 ml of internal standard was added into the solution and filled up to the mark with acetonitrile, thus the final concentration of internal standard hydrocortizone was always approximately 10 mg l^{-1}. The final concentrations of compounds in standard solutions were: estradiol (14.8 mg l^{-1}), estrone (4.7 mg l^{-1}), hydrocortizone (10.4 mg l^{-1}), propylparaben (13.9 mg l^{-1}) and methylparaben (26.7 mg l^{-1}). It was necessary to prepare fresh solutions every day.

2.4. Analytical repeatability testing

The sample of standard solution was injected six times into the chromatographic system. Peak retention times and peak areas were checked for all compounds using flow-rates 0.9 ml min^{-1} for conventional C_{18} column, 0.9 and 2.5 ml min^{-1} for 3.5 μm particle sized Eclipse XDB column and 0.9 and 1.2 ml min^{-1} for 1.8 μm particle sized Eclipse XDB column. Eclipse columns were tested at 25 and 40 °C. The mean values of retention times and peak areas were calculated and the standard deviations were determined.

3. Results and discussion

The chromatographic conditions originally developed for Estrogel analysis are described above. Under these conditions the analysis was repeated, all tested compounds (estradiol, methylparaben, propylparaben, hydrocortizone (IS) and estrone) were separated well. System suitability parameters (Table 1) meet all necessary criteria. Analytical run took about 11–12 min with typical back-pressure of about 24 MPa. That is quite a high for series of routine analyses. For these reasons, the use of high-throughput columns, which allows using of higher flow-rates, while the separation of compounds is unaffected or even better and the analytical run is much shortened has been proposed.

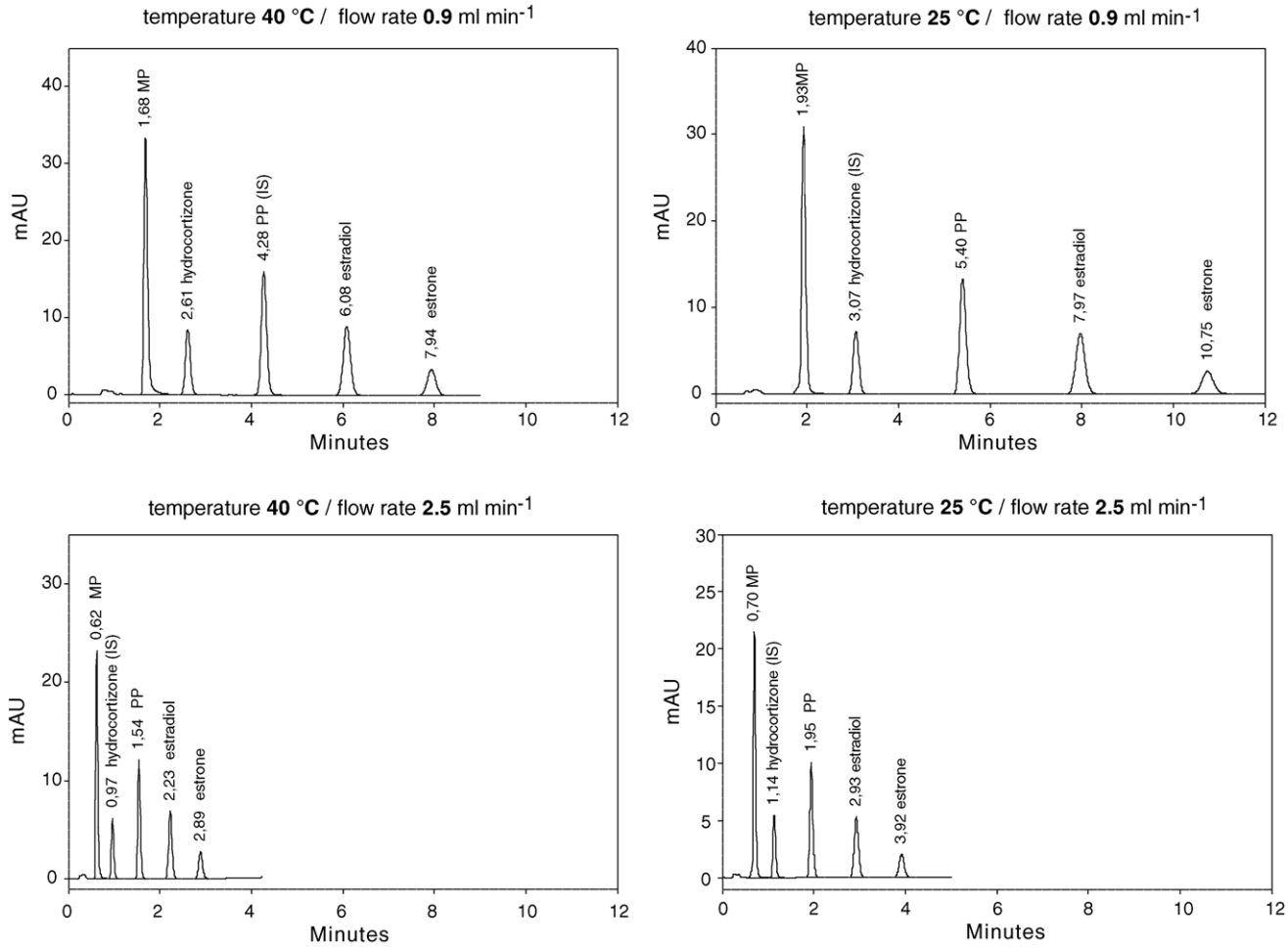

Fig. 3. Estrogel analysis on Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm) analytical column using different temperatures and flow-rates. MP: methylparaben, PP: propylparaben.

Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm) and Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm i.d., 1.8 μm) were used for these experiments, the same analytical conditions were applied, using normal flow-rate 0.9 ml min^{-1} and two extreme flow rates 2.5 ml min^{-1} for 3.5 μm particle sized columns and 1.2 ml min^{-1} for 1.8 μm particle sized columns. System suitability parameters and repeatability data were observed. The results can be seen in tables and in chromatograms—Figs. 3 and 4.

When using 1.8 μm particle columns, several instrument variables should be considered, so as to obtain optimum performance [25]. These are system volume, mobile phase flow-rate and data acquisition rate, which is probably the most important. With increasing column efficiency peak width decrease, therefore it is required to collect sufficient amount of data points of the peak. The rate should be higher than commonly used data collection every 1–2 s, for example 0.1 s. System volume reduction includes replacing standard 0.17 id mm tubing by 0.12 mm id tubing for all connections and replacing standard 13-μl detector cell by using Semi-Micro (8 μl) or Micro High Pressure (1.7 μl) cell with increased efficiency. The optimum flow-rate should be higher than for larger particle size columns. We were not able to try the modifications because the Shimadzu 2010 instrument is validated as a whole, therefore we tested 1.8 μm particle sized column at the common conditions. The results were excellent even without above mentioned modifications.

3.1. System suitability parameters

The results of system suitability testing could be seen in Table 1. Retention time as an index of compound quality is the most important parameter for the length of analysis. For all compounds using all tested conditions the retention times were shorter in case of tested Eclipse XDB columns than those conventional C_{18}. This means significant reduction of analysis duration, reducing solvent consumption and moreover, for routine series of analysis reduced time consumption.

For the definition of efficiency different parameters are used. In our study theoretical plate number and height equivalent of theoretical height were used. The later one is necessary in case of great differences in column length as

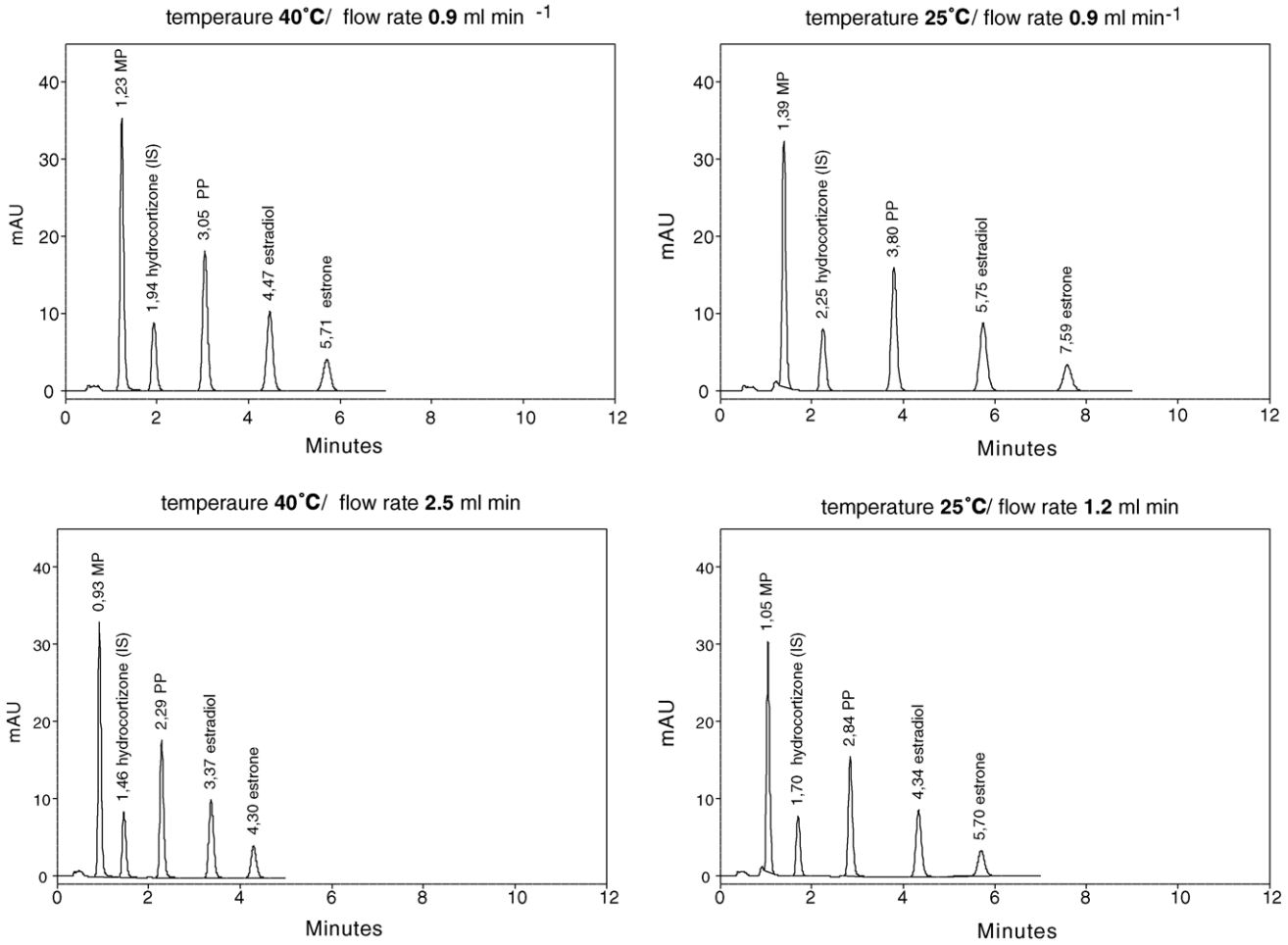

Fig. 4. Estrogel analysis on Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm i.d., 1.8 μm) analytical column using different temperatures and flow-rates. MP: methylparaben, PP: propylparaben.

Table 2
Analytical run repeatability—retention times

Column type	Methylparaben			Hydrocortizone (IS)			Propylparaben			Estradiol			Estrone		
	$\bar{x}$	S	R.S.D. (%)	$\bar{x}$	S	R.S.D. (%)	$\bar{x}$	S	R.S.D. (%)	$\bar{x}$	S	R.S.D. (%)	$\bar{x}$	S	R.S.D. (%)
ODS 5 μm 40 °C/0.9 ml min^{-1}	2.89	0.00	0.00	4.17	0.00	0.00	6.28	0.00	0.00	8.70	0.01	0.09	10.69	0.01	0.10
XDB 3.5 μm 40 °C/0.9 ml min^{-1}	1.69	0.01	0.30	2.61	0.00	0.00	4.28	0.00	0.00	6.09	0.00	0.08	7.94	0.01	0.07
XDB 3.5 μm 40 °C/2.5 ml min^{-1}	0.62	0.00	0.00	0.97	0.00	0.49	1.54	0.00	0.00	2.23	0.00	0.00	2.89	0.00	0.00
XDB 3.5 μm 25 °C/0.9 ml min^{-1}	1.93	0.00	0.00	3.07	0.00	0.00	5.40	0.00	0.07	7.97	0.00	0.05	10.76	0.01	0.07
XDB 3.5 μm 25 °C/2.5 ml min^{-1}	0.70	0.00	0.00	1.14	0.00	0.00	1.95	0.00	0.00	2.93	0.00	0.00	3.92	0.00	0.00
XDB 1.8 μm 40 °C/0.9 ml min^{-1}	1.23	0.00	0.00	1.94	0.00	0.00	3.05	0.00	0.00	4.47	0.00	0.00	5.71	0.00	0.07
XDB 1.8 μm 40 °C/1.2 ml min^{-1}	0.93	0.00	0.00	1.46	0.00	0.00	2.29	0.00	0.16	3.37	0.01	0.28	4.31	0.00	0.11
XDB 1.8 μm 25 °C/0.9 ml min^{-1}	1.39	0.00	0.27	2.25	0.00	0.00	3.80	0.00	0.00	5.75	0.00	0.08	7.59	0.00	0.06
XDB 1.8 μm 25 °C/1.2 ml min^{-1}	1.05	0.00	0.00	1.70	0.00	0.00	2.84	0.00	0.00	4.34	0.01	0.12	5.70	0.00	0.07

$\bar{x}$: mean retention time, S: standard deviation, R.S.D. (%): relative standard deviation, ODS 5: Discovery C_{18} (250 mm × 3.0 mm i.d.; 5 μm), XDB 3.5: Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm), XDB 1.8: Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm i.d., 1.8 μm).

Table 3
Analytical run repeatability—peak area

Column type	Methylparaben			Hydrocortizone (IS)			Propylparaben			Estradiol			Estrone		
	$\bar{x}$	S	R.S.D. (%)	$\bar{x}$	S	R.S.D. (%)	$\bar{x}$	S	R.S.D. (%)	$\bar{x}$	S	R.S.D. (%)	$\bar{x}$	S	R.S.D. (%)
ODS 5 μm 40 °C/0.9 ml min^{-1}	165034	314.28	0.19	72163	114.91	0.16	67139	118.58	0.18	113796	162.79	0.14	50100	51.15	0.10
XDB 3.5 μm 40 °C/0.9 ml min^{-1}	198087	213.08	0.11	65120	129.54	0.20	132617	273.84	0.21	99176	167.58	0.17	45036	107.51	0.24
XDB 3.5 μm 40 °C/2.5 ml min^{-1}	71367	186.90	0.26	23535	218.47	0.93	47836	270.34	0.57	35567	125.98	0.35	16147	82.79	0.51
XDB 3.5 μm 25 °C/0.9 ml min^{-1}	197308	770.40	0.39	64054	304.68	0.48	132244	139.16	0.11	98590	103.57	0.11	44802	185.02	0.41
XDB 3.5 μm 25 °C/2.5 ml min^{-1}	71259	550.04	0.77	23387	195.63	0.84	47329	30.07	0.06	35226	29.28	0.08	16036	29.31	0.18
XDB 1.8 μm 40 °C/0.9 ml min^{-1}	196267	655.27	0.33	64291	475.03	0.74	131600	35.94	0.03	97500	44.62	0.05	44242	249.90	0.56
XDB 1.8 μm 40 °C/1.2 ml min^{-1}	146613	518.73	0.35	48076	376.57	0.78	98731	374.44	0.38	73123	207.02	0.28	33347	148.17	0.44
XDB 1.8 μm 25 °C/0.9 ml min^{-1}	186990	196.42	0.11	63548	55.67	0.09	131030	112.55	0.09	97288	40.12	0.04	44119	77.90	0.18
XDB 1.8 μm 25 °C/1.2 ml min^{-1}	140993	398.32	0.28	47878	143.86	0.30	98110	87.92	0.09	72821	210.94	0.29	33046	140.22	0.42

$\bar{x}$: mean retention time, S: standard deviation. R.S.D. (%): relative standard deviation, ODS 5: Discovery C_{18} (250 mm × 3.0 mm i.d.; 5 μm), XDB 3.5: Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm), XDB 1.8: Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm i.d., 1.8 μm).

was ours, because theoretical plate number does not include the column length. Apparently, the efficiency of tested Eclipse XDB columns is incomparably higher in comparison with conventionally used C_{18} column.

Peak resolution describes the rate of compounds separation. It was satisfactory for all tested compounds. The value required by ICH is 1.5. Using the same conditions that mean flow-rate 0.9 ml min^{-1} (regardless the temperature) the resolution is even higher for tested Eclipse XDB columns comparing to conventional C_{18} column. Using extreme conditions, thus higher flow-rates (2.5 or 1.2 ml min^{-1}) the resolution decreases, but it is still sufficiently convenient.

Peak asymmetry is important for precise peak integration and thus for quantitative information. For all compounds the peak asymmetry is better in case of tested Eclipse XDB columns comparing to conventional C_{18} except of values for hydrocortizone. Anyway, this value is still meeting ICH requirements for validation, which recommend the value less than 1.5.

3.2. Analytical run repeatability

The repeatability of analytical run was tested in two levels—for retention time and for peak area repeatability. All results meet the criteria necessary for validation according ICH requirements that means relative standard deviation is not higher than 1%. For the retention times—Table 2, the highest R.S.D. 0.49% was in case of hydrocortizone retention time using Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm) at flow-rate 2.5 ml min^{-1} and temperature 40 °C. For most of analysis the R.S.D. is 0.00% or very close to 0.00%. This value increases with retention time of compounds. Thus for estrone as the last compound in chromatogram it is usually more than 0.06%, but it is not higher than 0.11%. The R.S.D. values for retention times are similar for all types of columns.

Concerning peak area repeatability—Table 3, the diversity of R.S.D. values was much greater. The highest R.S.D. 0.93% was again in case of hydrocortizone peak area using Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm) at flow-rate 2.5 ml min^{-1} and temperature 40 °C. For the methylparaben the R.S.D. values were found inside the range 0.11–0.77%, for hydrocortizone 0.09–0.93%, for propylparaben 0.03–0.57%, for estradiol 0.04–0.35% and for estrone 0.10–0.56%. Again, the R.S.D. values for peak areas are similar for all types of columns, there is not very significant difference.

3.3. Solvent consumption, system maintenance

Regarding analysis time and solvent consumption – Table 4 – the choice of final chromatographic conditions must be a compromise. The higher is the flow-rate, the higher is usually solvent consumption as well. Thus the shortest analyses time we were able to perform with Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm) at flow-rate 2.5 ml min^{-1} and temperature 40 °C with the solvent consumption 8.75 ml. Generally, from the point of view of analysis time, it is unambiguously the most advantageous to use Zorbax Eclipse XDB 3.5 μm particle sized column at flow-rate 2.5 ml min^{-1} or Zorbax Eclipse XDB 1.8 μm particle sized column at 1.2 ml min^{-1} and at 40 °C temperature. With the regard of solvent consumption, the most suitable is to use Zorbax Eclipse XDB 1.8 μm particle sized column at any tested mode.

Table 4
Solvent consumption, system maintenance

Column type	Solvent consumption (ml)	Duration of analysis (min)	Column back-pressure (MPa)
ODS 5 μm 40 °C/0.9 ml min^{-1}	9.90	11.0	26.0
XDB 3.5 μm 40 °C/0.9 ml min^{-1}	7.65	8.5	6.4
XDB 3.5 μm 40 °C/2.5 ml min^{-1}	8.75	3.5	17.4
XDB 3.5 μm 25 °C/0.9 ml min^{-1}	9.90	11.0	8.3
XDB3.5 μm 25 °C/2.5 ml min^{-1}	11.25	4.5	23.0
XDB 1.8 μm 40 °C/0.9 ml min^{-1}	5.40	6.0	13.4
XDB 1.8 μm 40 °C/1.2 ml min^{-1}	5.40	4.5	17.7
XDB 1.8 μm 25 °C/0.9 ml min^{-1}	7.20	8.0	17.5
XDB 1.8 μm 25 °C/1.2 ml min^{-1}	7.20	6.0	23.2

ODS 5: Discovery C_{18} (250 mm × 3.0 mm i.d.; 5 μm), XDB 3.5: Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm), XDB 1.8: Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm i.d., 1.8 μm).

Performing the series of routine analyses it is important to consider also column maintenance. That means choosing of appropriate mobile phases, careful system washing and application of adequate flow-rates with the regards of column and system properties. The analytical conditions for tested columns were chosen considering the method to be frequently used in routine analysis. Therefore it is convenient to lower the column back-pressure as much as possible. High throughput columns are made for fast analyses performing, which saves time and analytical system as well. The column length is shorter than that conventional, thus the column back-pressure is not so high as is was proven in this study. Thus, for the shortening analysis time with regard to the back-pressure, Zorbax Eclipse XDB analytical columns are more convenient as it could be seen in Table 4.

4. Conclusion

A comparison of conventional C_{18} analytical column Discovery C_{18} (250 mm × 3.0 mm i.d., 5.0 μm) and novel types of C_{18} columns Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm) and Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm

i.d., 1.8 μm) was made. All types of columns were able to separate tested compounds well with sufficient resolution and peak asymmetry, but they differed in analysis time and column back-pressure.

The data presented in this article show explicit advantages of the new types of analytical columns comparing to conventional "long" C_{18} columns with 5 μm particles. These advantages are particularly a significant reduction of analysis time, which means reduction in solvent consumption as well. Retention data including peak asymmetry and peak resolution values are preferable for Zorbax Eclipse XDB-C_{18} (75 mm × 4.6 mm i.d., 3.5 μm) and Zorbax Eclipse XDB-C_{18} (50 mm × 4.6 mm i.d., 1.8 μm) comparing to conventional C_{18}. The improvement of efficiency is apparently seen from the height equivalent of theoretical plate values. It is clearly seen, that high through-put analytical columns are more convenient and efficient performing complex analysis of pharmaceutical preparation Estrogel gel.

Generally, they can be of substantial importance especially in pharmaceutical industry, where it is necessary to perform many analysis in the area of quality control of pharmaceuticals and during stability studies as well.

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