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Comparison of performance of C18 monolithic rod columns and conventional C18 particle-packed columns in liquid chromatographic determination of Estrogel and Ketoprofen gel

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Abstract

The performance of monolithic HPLC columns Chromolith™ (made by Merck, Germany) and conventional C18 columns Discovery (Supelco, Sigma–Aldrich, Prague, Czech Republic) was tested and the comparison for two topical preparations Ketoprofen gel and Estrogel gel was made. The composition of mobile phases – for Ketoprofen analysis a mixture of acetonitrile, water and phosphate buffer adjusted to pH 3.5 (40:58:2) and for Estrogel analysis a mixture of acetonitrile, methanol, water (23:24:53) – was usually not optimal for analyses at all types of columns. Thus an adjustment of components ratio was necessary for sufficient resolution of the compounds analysed. Various flow rates (1.0–5.0 ml/min) and mobile phases (usually increasing ratio of water content) were applied. Determination of active substances, preservatives and impurities and comparison of retention times and system suitability test parameters was accomplished. For Estrogel gel, following chromatographic conditions were found: using Chromolith Flash RP-18e monolith column, mobile phase was acetonitrile, methanol, water (13:24:63, v/v/v) and flow-rate 3.0 ml/min. Using monolith column ChromolithSpeedROD RP-18e, the mobile phase was acetonitrile, methanol, water (18:24:58, v/v/v) and flow-rate 4.0 ml/min. For the monolith column Chromolith Performance RP-18e, the mobile phase was acetonitrile, methanol, water (23:24:53, v/v/v), flow-rate 3.0 ml/min. Analysis of Ketoprofen gel gave the best results using following analytical conditions: for monolith column Chromolith Flash RP-18e, mobile phase as a mixture of acetonitrile, water, phosphate buffer pH 3.5 (30:68:2, v/v/v) was used, at flow-rate 2.0 ml/min. For ChromolithSpeedROD RP-18e monolith column, acetonitrile, water, phosphate buffer pH 3.5 (35:63:2, v/v/v) was used as a mobile phase at flow-rate 3.0 ml/min. Chromolith Performance RP-18e gave the best results using mobile phase acetonitrile, water, phosphate buffer pH 3.5 (30:68:2, v/v/v) at the flow-rate 5.0 ml/min. It was proved that monolith columns, due to their porosity and low back-pressure, can save analysis time by about a factor of three with sufficient separation efficiency. Thus, for example 11 min long analysis can be performed in 4 min with comparable results.

Keywords: Monolithic columns; Ketoprofen; Estrogel

1. Introduction

Nowadays the most challenging trend in liquid chromatography (and for the near future as well) is the development of new sorbents, which are able to separate efficiently complicated substances, e.g. polar or basic. Such sorbents should be able to work in a wide pH range and should perform analysis as fast as possible while sufficient separation, method sensitivity and selectivity remain unaffected.

One of these novel types of sorbents is a monolithic silica (analytical columns Chromolith™ Merck) [1]. They

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mark out different structure comparing to conventional silica. While the typically used columns are filled with small silica spherical particles, monolithic columns contain a special silica (or another material), which is not formed by particles. They are made by sol–gel technology, which enables formation of highly porous material, containing macropores and mesopores in its structure. Such an LC column consists of a single rod of silica based material with two kinds of pores. The large pores (typically 2 μm) are responsible for a low flow resistance and therefore allow the application of high eluent flow-rates, while the small pores (about 12 nm) ensure sufficient surface area for separation efficiency. Due to this facts, higher flow rates can be used while the resolution of silica rod column is much less affected in comparison to particulate materials after increasing the flow-rate and column back-pressure is still low. Another practical advantage is a short time needed for column equilibration when a mobile phase gradient is used. It is also possible to apply flow-rate gradient as well.

Silica is often used for preparation of monolithic columns. It could be simple silica only used in a normal mode [2–4], or it can be modified by ODS group [5,6] or by pentafluorophenyl-propyl-dimethyl group, pentafluorophenyl, 3,3,3-trifluoropropyl, *n*-octamethyl, perfluorohexyl or aminopropyl group for reversed phase chromatography [7]. Nevertheless, silica is not the only material used for preparation of monolithic columns. They can be made also from various polymers like widely used polymethacrylates [8–13], methacryloxypropyltrimethoxysilane [14] cationic stearylacrylate [15], acrylamides [16], poly(styrene-divinybenzene) [17–19], vinylpyridines, vinylpyrrolidone, vinyl acetate [20] or urea-formaldehyde resin for affinity chromatography [21]. For the purpose of enantiometric separation silica columns can be modified with chiral selectors, such as L-phenylalaninamide, L-alaninamide or L-prolinamide [22]. All the monolithic chromatographic columns can be classified into three categories: rods—could be either end-capped or not (including analytical, capillary columns and microchips), disks and tubes [20]. Although monolithic columns are mostly prepared by laboratories themselves, they are commercially available from several manufactures as well.

So far the practical use of monolithic columns has not been as wide as one should expect, in spite of all their advantages. There are a lot of papers concerning monolithic columns, but most of the articles published describe mainly preparation and testing of monolithic columns [12,16,23–26]. Only a few works deal with practical application of monolithic columns. They mostly include bio-analytical area [9–11,27–37]. The others refer to β-caroten isomers separation using six coupled monolith columns and an ODS particulate one [38], heroin acidic and neutral impurities determination [39], enantiometric separation of propranolol isomers [40] or about pesticides and their metabolites determination [41]. One paper was dealing with repeatability and reproducibility of retention data and band profiles of monolithic columns made by Merck [42]. A lot of methods use capillary monolithic columns in connection with CEC [15,16,19,24–27,31,33–35,37,39,40]. Thus it seems to be a little easier to get good results using self-made monolithic materials prepared within capillary in CEC mode than in LC-mode and that rod monolith columns have a relatively rare practical utilization in spite of all their advantages.

The aim of this work was to compare performance of conventional C18 (Discovery^{TM} C18) stationary phases using methods which have been previously developed and validated, with two new methods applying monolithic columns. The first method compared separation and determination of active substances, impurities and preservatives in topical pharmaceutical formulations Estrogel gel (estradiol, methylparaben, propylparaben, estrone), the second in formulation Ketoprofen gel (ketoprofen, methylparaben, propylparaben, impurity A (3-acetylbenzophenone) and impurity C (2-(3-carboxyphenyl)propionic acid) according valid pharmacopoeia.

2. Experimental

2.1. Chemicals and reagents

Working standards of estradiol, ketoprofen, estrone, methylparaben, ethylparaben (internal standard), 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).

3-Acetylbenzophenone–Ketoprofen impurity A CRS and 2-(3-carboxyphenyl)propionic acid–Ketoprofen impurity C CRS were obtained from Council of Europe (European Pharmacopoeia Strasbourg, France).

Phosphoric acid 85% p.a. and dihydrogen potassium phosphate 99.5% p.a., were purchased from Merck (Darmstadt, Germany).

Acetonitrile, Supragradient, was obtained from Biotech (Scharlau 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 (Shimadzu, Kyoto, 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 Ketoprofen gel were as follows: analyses were performed using analytical column Discovery^{TM} C18 (125 × 4.0

i.d., 5 μm) at ambient temperature. Mobile phase was a mixture of acetonitrile, water and phosphate buffer adjusted to pH 3.5 (40:58:2), it was pumped isocratically at the flow-rate 1.0 ml/min. Injection volume was 10 μl and detection of all compounds was accomplished at 233 nm. Ethylparaben was used as an internal standard for quantitation.

Original chromatographic conditions for determination of Estrogel gel were: analyses were performed using analytical column Discovery^{TM} C18 (250 × 3.0 i.d., 5 μm) at column oven temperature 40 °C. Mobile phase was a mixture of acetonitrile, methanol, water (23:24:53), it was pumped isocratically at the flow-rate 0.9 ml/min. Five microliters of sample was injected into analytical column, detection of analytes was accomplished at 225 nm. Hydrocortizone was used as an internal standard for quantitation [43].

Firstly, the two pharmaceutical formulations were tested using original conditions. Afterwards the conditions were applied to monolithic columns. Three types of columns were tested: Chromolith Flash RP-18e (25 × 4.6 mm i.d.), ChromolithSpeedROD RP-18e (50 × 4.6 mm i.d.) and Chromolith Performance RP-18e (100 × 4.6 mm i.d.), all made by Merck (Darmstadt, Germany). There was a need to change mobile phase ratio because the analyses were too fast and separation of individual compounds was not satisfactory. Different ratios of mobile phases were tested-increasing the amount of water content in mobile phase was usually sufficient solution for good separation. Optimal conditions for analysis time and compounds separation were chosen with regard to solvent consumption as well. Retention times and System Suitability Test parameters (resolution, number of theoretical plates and asymmetry) were compared.

2.3. Reference standard preparation

The stock solutions of internal standards were prepared by dissolving 50 mg of hydrocortizone, ethylparaben, respectively, in 100 ml of acetonitrile. 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 hydrocortizone stock solution was added and the flask was made up to the volume with acetonitrile.

A Reference standard solution for Ketoprofen gel analysis was prepared in 100 ml volumetric flask by dissolving of 62.5 mg of ketoprofen, 2.5 mg of methylparaben, 1.25 mg of propylparaben, 0.5 mg of impurity A and 0.5 mg of impurity C in acetonitrile. Finally 2.0 ml of internal standard ethylparaben stock solution was added and the flask was made up to the volume with acetonitrile.

Working solutions of internal standards were prepared by diluting 10.0 ml of the internal standard stock solution in acetonitrile to a volume of 500.0 ml. Thus the final concentration of internal standard hydrocortizone or ethylparaben was always approximately 10 mg/l. It was necessary to prepare fresh solutions every day.

2.4. Sample preparation

Sample preparation procedure was – due to the similar composition of excipients in both gels – the same for both topical preparations. About 0.5 g of topical Estrogel HBF gel or Ketoprofen gel (which corresponds to 0.3 mg of estradiol or 12.5 mg of ketoprofen) was accurately weighed and was transferred into 50.0 ml centrifuge tube. Twenty milliliters of internal standard working solution in acetonitrile (10 mg/l of hydrocortizone in acetonitrile or 10 mg/l of ethylparaben in acetonitrile, respectively) were added. This mixture was sonicated for 10 min and then centrifuged for 15 min at 1300 × *g* (laboratory centrifuge EBA 21, Hettich, Tutlingen, Germany). The supernatant was injected directly into the chromatographic system.

2.5. Mobile phase preparation

Mobile phase was prepared by simple mixing of individual components acetonitrile, methanol and water (23:24:53) or acetonitrile, water and phosphate buffer pH 3.5 (40:58:2), respectively. Afterwards it was filtered using Millipore filtration device.

Phosphate buffer pH 3.5 was prepared following prescriptions of European Pharmacopoeia. 68.8 g of potassium dihydrogenphosphate was dissolved in water R and diluted up to 1000.0 ml. pH of this solution was adjusted by phosphoric acid R. Two milliliters of 6.88% buffer were used for mobile phase preparation, thus the final concentration in aqueous part was 4.36%.

3. Results and discussion

3.1. Ketoprofen

The original method for Ketoprofen gel determination is stated above and under these conditions all tested compounds (ketoprofen, methylparaben, propylparaben, ethylparaben (IS), impurity A and impurity C) were separated well as it could be seen at Fig. 1. System suitability parameters (Table 1) meet all necessary criteria. Analytical run took 9 min, typical back-pressure was about 10 MPa.

The original method was transferred to a system with monolith column. Different lengths of Chromolith^{TM} C18 columns were tested: Chromolith Flash RP-18e (25 × 4.6 mm i.d.), ChromolithSpeedROD RP-18e (50 × 4.6 mm i.d.) and Chromolith Performance RP-18e (100 × 4.6 mm i.d.). There was a need to adjust chromatographic conditions in order to get better results for each column. While the column oven temperature and detection wavelength remain unaffected, different mobile phase compositions (usually increasing of water content) and flow-rates in a range 1.0–5.0 ml/min were tested. The best results were chosen and compared to original conditions. System suitability parameters, analysis time,

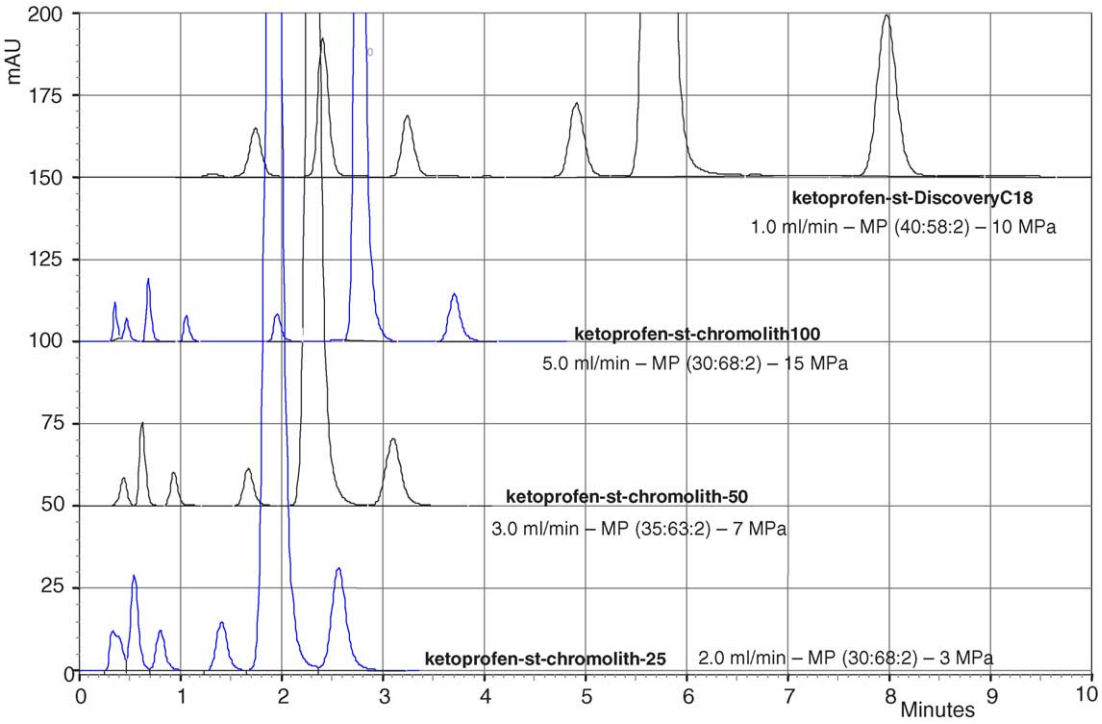

Fig. 1. *Chromatogram* 1: A comparison of performance of individual monolith columns and conventional column using analytical method for Ketoprofen gel. (*Eluting peaks*: 2-(3-Carboxyphenyl) propionic acid, methylparaben, ethylparaben (IS), propylparaben, ketoprofen, 3-acetybenzophenone.) *Note*: The same time scale is used for all chromatograms.

Table 1
A comparison of retention times and SST parameters for analytical column Discovery™ C18 and monolithic column Chromolith™ testing analytical method for Ketoprofen gel

Substance	t_r (min)	w (min)	N	H (μm)	R_{ii}	T	Analytical conditions
Impurity C	1.75	0.13	960	130.20	1.95	1.00	Analytical column: Discovery™ C18 (125 × 4.0 mm i.d., 5 μm); mobile phase: acetonitrile, water, phosphate buffer pH 3.5 (40:58:2, v/v/v); flow-rate: 1.0 ml/min; Column oven temperature: 25 °C; Injection volume: 10 μl
Methylparaben	2.41	0.11	2280	54.80	3.08	1.25	
Ethyparaben-IS	3.24	0.13	3294	37.90	3.90	1.27	
Propylparaben	4.92	0.16	5158	24.20	6.70	1.03	
Ketoprofen	5.70	0.19	5077	24.60	2.64	1.18	
Impurity A	7.97	0.21	7981	15.70	6.72	1.13	
Impurity C	0.33	0.14	10	2500.00	0.00	1.11	Monolith column: Chromolith Flash RP-18e (25 × 4.6 mm i.d.); mobile phase: acetonitrile, water, phosphate buffer pH 3.5 (30:68:2, v/v/v); flow-rate: 2.0 ml/min; column oven temperature: 25 °C; injection volume: 10 μl
Methylparaben	0.55	0.08	217	115.20	0.76	0.83	
Ethyparaben-IS	0.81	0.10	363	68.90	1.62	1.14	
Propylparaben	1.42	0.12	728	34.30	3.20	1.13	
Ketoprofen	1.93	0.16	801	31.20	2.14	1.17	
Impurity A	2.57	0.16	1427	17.50	2.32	1.12	
Impurity C	0.44	0.08	155	322.60	0.00	0.95	Monolith column: ChromolithSpeedROD RP-18e (50 × 4.6 mm i.d.); mobile phase: acetonitrile, water, phosphate buffer pH 3.5 (35:63:2, v/v/v); flow-rate: 3.0 ml/min; column oven temperature: 25 °C; injection volume: 10 μl
Methylparaben	0.63	0.06	473	105.70	1.43	0.88	
Ethyparaben-IS	0.94	0.07	798	62.70	2.55	1.13	
Propylparaben	1.68	0.10	1368	36.50	4.65	1.33	
Ketoprofen	2.31	0.14	1457	34.30	2.99	1.14	
Impurity A	3.10	0.16	1943	25.70	3.03	1.12	
Impurity C	0.47	0.05	344	290.70	1.26	1.10	Monolith column: Chromolith Performance RP-18e (100 × 4.6 mm i.d.); mobile phase: acetonitrile, water, phosphate buffer pH 3.5 (30:68:2, v/v/v); flow-rate: 5.0 ml/min; column oven temperature: 25 °C; injection volume: 10 μl
Methylparaben	0.68	0.05	813	123.00	2.10	1.38	
Ethyparaben-IS	1.06	0.06	1611	62.10	3.70	1.64	
Propylparaben	1.98	0.08	3043	32.90	2.46	1.36	
Ketoprofen	2.77	0.11	3641	27.50	1.17	1.30	
Impurity A	3.71	0.12	6005	16.70	1.39	1.25	

system back-pressure and mobile phase consumption were checked.

The best results with Chromolith Flash RP-18e (25 × 4.6 mm i.d.) analytical column were reached with mobile phase composition acetonitrile, water and phosphate buffer adjusted to pH 3.5 (30:68:2) at flow-rate 2.0 ml/min (Table 1, Fig. 1). Analytical run took about 3–4 min and the back-pressure – only about 3 MPa – was much lower comparing to conventional C18 column. For this column, the separation efficiency was found to be not sufficient, probably due to the shortage of the column. Even after testing of different mobile phase compositions, no better peak resolutions were achieved. Much better result were obtained only with longer monolith columns.

ChromolithSpeedROD RP-18e (50 × 4.6 mm i.d.) show the best separation using mobile phase acetonitrile, water and phosphate buffer adjusted to pH 3.5 (35:63:2) at flow-rate 2.0 ml/min (Table 1, Fig. 1). Analytical run took not more than 3.5 min, with the back-pressure less than 7 MPa.

For Chromolith Performance RP-18e (100 × 4.6 mm i.d.) the best results were found with mobile phase composition acetonitrile, water and phosphate buffer adjusted to pH 3.5 (30:68:2) at flow-rate 5.0 ml/min (Table 1, Fig. 1). The analytical run took about only 4.5 min and the back-pressure was about 15 MPa. This is much higher than in previous measurements using shorter columns and is caused by the longer length of the column. On the contrary, considering the high flow-rate used in this case (5.0 ml/min) such a back-pressure is not a problem and excellent performance of the column was achieved.

From all the results it can be seen that the major advantage of monolith columns is reduction in a run time while the separation efficiency is affected less comparing to particulate columns. Decreasing of back-pressure on analytical column is useful as well, especially for extended column life-time.

3.2. Estradiol

Originally, Estrogel analysis follows the conditions described earlier [43]. Under these conditions all tested compounds (estradiol, methylparaben, propylparaben, hydrocortisone (IS) and estrone) were separated well (Fig. 2). System suitability parameters (Table 2) meet all necessary criteria. Analytical run took about 11–12 min, while the typical back-pressure was about 24 MPa being a relatively high for series of routine analyses.

The method developed on conventional column was transferred to a monolith column, using the same lengths of Chromolith^{TM} C18 column. Ambient column oven temperature and elevated temperature used at original method were modified while the detection wavelength remained unchanged. Different mobile phase compositions (usually increasing of water content) and flow-rates in a range 1.0–5.0 ml/min were tested. The best results were chosen and compared to original conditions. System suitability parameters, analysis time, system back-pressure and mobile phase consumption were checked.

The best results at elevated temperature 40 °C with Chromolith Flash RP-18e (25 × 4.6 mm i.d.) analytical column were reached with mobile phase composition acetonitrile, methanol, water (13:24:63) at flow-rate 3.0 ml/min (Table 2, Fig. 2). Analytical run took about 3.5 min and the back-pressure was much lower comparing to conventional particulate C18 column, less than 10 MPa.

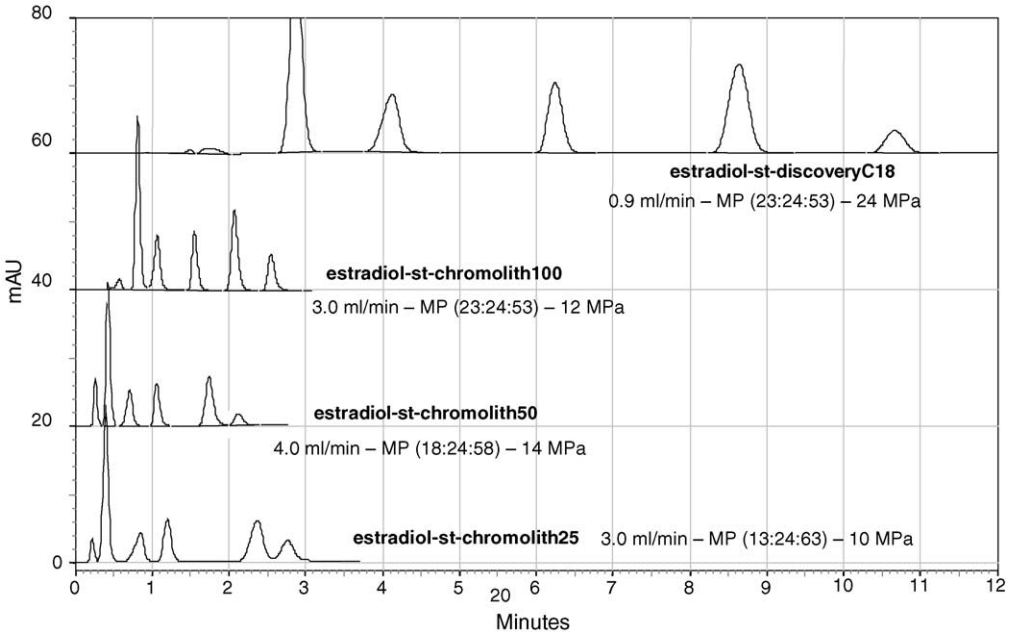

Fig. 2. *Chromatogram* 2: A comparison of performance of individual monolith columns and conventional column using analytical method for Estrogel gel. (*Eluting peaks*: Methylparaben, hydrocortizone (IS), propylparaben, estradiol, estrone.). *Note*: The same time scale is used for all chromatograms.

Table 2
A comparison of retention times and SST parameters for analytical column Discovery™ C18 and monolithic column Chromolith™ testing analytical method for Estrogel gel

Substance	t_r (min)	w (min)	N	H (μm)	R_{ii}	T	Analytical conditions
Methylparaben	2.88	0.16	1721	145.30	3.38	1.42	Analytical column: Discovery™ C18 (250 × 3.0 mm i.d., 5 μm); mobile phase: acetonitrile, methanol, water (23:24:53, v/v/v); flow-rate: 0.9 ml/min; column oven temperature: 40 °C; injection volume: 5 μl
Hydrocortizone-IS	4.13	0.24	1639	152.50	3.58	1.07	
Propylparaben	6.24	0.21	5048	49.50	5.48	1.11	
Estradiol	8.64	0.29	5085	49.20	5.74	1.02	
Estrone	10.67	0.29	7772	32.20	4.18	1.04	
Methylparaben	0.40	0.07	148	168.90	1.58	1.00	Monolith column: Chromolith Flash RP-18e (25 × 4.6 mm i.d.); mobile phase: acetonitrile, methanol, water (13:24:63, v/v/v); flow-rate: 3.0 ml/min; column oven temperature: 40 °C; injection volume: 5 μl
Hydrocortizone-IS	0.85	0.13	214	116.80	2.47	1.36	
Propylparaben	1.20	0.11	683	36.60	1.68	1.10	
Estradiol	2.38	0.18	932	26.80	4.75	0.97	
Estrone	2.77	0.18	1318	19.00	1.27	0.95	
Methylparaben	0.42	0.06	248	201.60	1.66	1.08	Monolith column: ChromolithSpeedROD RP-18e (50 × 4.6 mm i.d.); mobile phase: acetonitrile, methanol, water (18:24:58, v/v/v); flow-rate: 4.0 ml/min; column oven temperature: 40 °C; injection volume: 5 μl
Hydrocortizone-IS	0.71	0.08	339	147.50	2.16	1.00	
Propylparaben	1.06	0.08	911	54.90	2.38	1.06	
Estradiol	1.74	0.11	1282	39.00	4.08	1.14	
Estrone	2.13	0.12	1723	29.00	1.92	1.04	
Methylparaben	0.81	0.06	950	105.30	2.03	1.17	Monolith column: Chromolith Performance RP-18e (100 × 4.6 mm i.d.); mobile phase: acetonitrile, methanol, water (23:24:53, v/v/v); flow-rate: 3.0 ml/min; column oven temperature: 40 °C; injection volume: 5 μl
Hydrocortizone-IS	1.07	0.08	879	113.80	2.06	1.20	
Propylparaben	1.55	0.07	2349	42.60	3.58	1.21	
Estradiol	2.07	0.09	2743	36.50	3.63	1.31	
Estrone	2.55	0.10	3710	27.00	2.91	1.33	

ChromolithSpeedROD RP-18e (50 × 4.6 mm i.d.) shows the best separation using mobile phase acetonitrile, acetonitrile, methanol, water (18:24:58) at flow-rate 4.0 ml/min (Table 2, Fig. 2). An analytical run took 3 min and the back-pressure was about 14 MPa. That means the analytical run time was decreased four times, while the back-pressure was still quite low considering such a high mobile phase flow-rate.

For Chromolith Performance RP-18e (100 × 4.6 mm i.d.) the best results were found with mobile phase composition acetonitrile, methanol, water (23:24:53) at flow-rate 3.0 ml/min (Table 2, Fig. 2), which means the same composition, only increasing the flow-rate was sufficient for optimal compounds separation. Analytical run took about 3 min which is four times shorter compared to the original method. The back-pressure was about 12 MPa, which is two times less in contrast to particulate C18 column.

Comparing to conventional particulate ODS columns it was possible to perform Estradiol determination using monolith columns at ambient temperature (25 °C) as well. Discovery™ C18 (250 × 3.0 i.d., 5 μm) did not allow such an analysis, because the column back-pressure was too high (about 30 MPa). Of course it is possible to perform analysis under these conditions, but it decreases column life-time and also there is a greater possibility of exceeding the maximum pressure. When using monolith columns even at ambient temperature the back-pressure decreased, thus the separation was possible. Analytical runs were about two times shorter comparing to original method. However, the results at elevated temperature (40 °C) were still better, thus the analyses were finally performed at these conditions.

It was proved that the major advantage of monolith columns is reduction in a run time, while separation efficiency remains unaffected or it is even better. It is important especially when series of analyses are done, e.g. stability testing of pharmaceuticals or quality control during manufacturing process. Thus, decreasing analysis time is one of the most important aspects of method development.

Considering chromatographic conditions, there are always limitations. Column oven temperature is influenced by sorbent type and its stability at elevated temperatures so there is the upper limit which can not be exceeded to prevent sorbent damage. Change of mobile phase composition is sometimes very helpful. The limitations are given by sorbent pH stability range, which is very different for particular sorbents. Sometimes there could be a problem of mobile phase components precipitation, therefore the mobile phase adjustment is not always possible. The influence of flow-rate of mobile phase is also important, but it is limited by stationary phase resistance again. Chromatographic system usually can withstand pressures up to 40 MPa, but there could be some differences among individual analytical columns. Decreasing the column back-pressure is also important for extended column life-time.

With respect to the above mentioned aspects, the only optimal solution seems to be the choice stationary phase which is pH resistant, enables operating at increased temperatures or gives low resistance to mobile phase flow and have a great separation efficiency. As it was proven, monolith columns meet all these criteria so they are an ideal choice for repeated analyses, especially at quality control laboratory in pharmaceutical companies.

4. Conclusion

A comparison of conventional methods using Discovery^{TM} C18 particle-packed columns and methods using novel monolithic columns Chromolith^{TM} C18 of different lengths was made. Both types of columns were able to separate tested compounds well with sufficient resolution and peak asymmetry, but they differ in analysis time and column back-pressure significantly. While the conventional particulate columns perform analytical run at longer time with higher back-pressure, monolith columns are more convenient. They enable performing analytical run under lower back-pressure at shorter time. This was verified using two analytical methods for evaluation of pharmaceutical preparations Ketoprofen gel and Estrogel gel.

Monolithic LC columns are a useful means of increasing the separation efficiency per unit time, which can be achieved by increasing mobile phase flow. The fact that consumption of mobile phase per unit time is much higher comparing to conventional methods is compensated by the much shorter analyses times. That means that total solvent consumption per analysis is comparable with that of conventional method. If it is still too high, it can be compensated by using solvent saver.

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