Turk J Chem
30 (2006) , 543 – 552.

Performance Comparison between Monolithic C18 and Conventional C18 Particle-Packed Columns in the Liquid Chromatographic Determination of Propranolol HCl

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Received 24.01.2006

Monolithic and conventional particle-packed columns were applied for the determination of propranolol hydrochloride in the presence of its 2 main degradation products, 3-(1-naphthyloxy)-propane-1,2-diol and 4-isopropyl-1,7-bis-(1-naphthyloxy)-4-azaheptane-2,6-diol. The separations were investigated on monolithic columns at flow rates from 1 to 9 mL/min. Fast and efficient separation was obtained by monolithic columns. The analysis time was decreased by about 5-fold on monolithic columns at a flow rate of 4 mL/min, while maintaining sufficient resolution between propranolol and its degradation products. The method was validated using a set of 3 monolithic columns and compared to a conventional (Superspher) C18 column. The precision for both retention time and peak area was investigated over a wide concentration range (0.002- 1 mg/mL) and found to be equal or slightly better on Chromolith Performance compared to the conventional column. Batch to batch reproducibility of the Chromolith Performance columns (n = 3) was also calculated. The RSDs % equal 0.66% for retention time and ranged from 0.45% to 1.12% for peak areas. Practical parameters including the pressure drop, plate height, retention time and resolution of monolithic columns were compared to those of a conventional (Superspher) C18 column. The detection and quantitation limits on monolithic columns at both flow rates (1 and 4 mL/min) were 0.012 and 0.04 μg/mL, compared with 0.061 and 0.2 μg/mL on the conventional column. The method showed good linearity and recovery and was found to be suitable for the analysis of propranolol hydrochloride formulations.

Key Words: Monolithic columns, Chromolith Performance, propranolol hydrochloride, performance, validation, degradation products, method transfer, batch reproducibility.

Introduction

Propranolol hydrochloride, (RS)-1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride, is a non-selective beta-adrenergic blocking agent widely used in the treatment of hypertension, angina pectoris and

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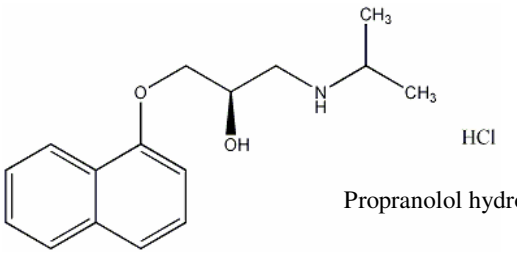
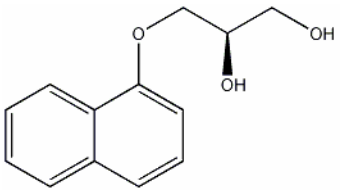
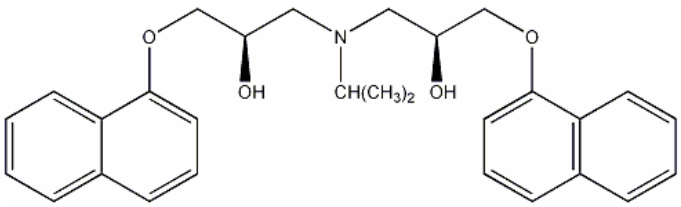
cardiac arrhythmias. The main degradation products of this drug include 3-(1-naphthyloxy)-propane-1,2-diol and 4-isopropyl-1,7-bis-(1-naphthyloxy)-4-azaheptane-2,6-diol[1]. The structures of propranolol hydrochloride and its 2 main degradation products are shown in Figure 1. Many methods have been reported for the determination of propranolol applying high performance liquid chromatography[2−6]. GC-MS[7]. UV spectrophotometric[8], cerimertric[9], phosphorimetric[10], voltammetric[11] and fluorimetric[12] methods have also been described. Only one method has been reported for the determination of impurities in propranolol. In this method, dynamically modified silica was used with a total run time of about 20 min[13].



Propranolol hydrochloride



Degradation product a
3-(1-naphthyloxy) propane-1, 2-diol



Degradation product b
4-isopropyl-1, 7-bis (1-naphthyloxy)-4-azaheptane-2,6-diol

Figure 1. Structure of propranolol hydrochloride and its 2 degradation products.

Monolithic silica columns are packed with a single piece of silica gel into a straight rod of highly porous silica with a bimodal pore structure referred to as macroporous and mesoporous[14]. The large macropores are responsible for a low flow resistance and therefore allow the application of high eluent flow-rate, while the small pores ensure sufficient surface area (300 m^2/g approximately) for separation efficiency. Monolithic columns also have a significantly higher total porosity compared to conventional particulate columns, over 80% vs. ca. 65%, respectively[15]. In spite of these promising characteristics, few analytical applications have been developed using monolithic columns, and the transferability of analytical methods from conventional particle-packed to monolithic columns is still in discussion. It has also been concerned that more investigations are still needed to ensure the applicability of these new silica type columns.

The aim of this work was to evaluate the chromatographic properties of commercial Chromolith Performance RP-18 HPLC columns for the analysis of propranolol, including separation from its 2 degradation products. Furthermore, the performance of this column will be compared to that of a conventional C18 column, starting from a pharmacopoeial method described for the determination of propranolol using a conventional C18 HPLC column[1]. Monolithic silica based columns will be investigated as a possibility for reducing separation time in reversed phase HPLC without significant sacrifice of efficiency or resolution. Batch to batch reproducibility for 3 monolithic columns will also be estimated.

Experimental

Chemicals and reagents

Acetonitrile HPLC grade and propranolol hydrochloride 99% were purchased from Acros Organics (Belgium). Methanol HPLC grade was obtained from Fisher Scientific (UK). Sulfuric acid and sodium lauryl sulfate were purchased from Merck (Darmstadt, Germany). Tetrabutylammonium dihydrogen phosphate was obtained from Sigma-Aldrich (Switzerland). All substances were at least of analytical grades. Bi-distilled water was used throughout.

Instrumentation

Analyses were performed on a MERCK Hitachi HPLC system consisting of a solvent pump (model L 6200 A), an autosampler (AS 2000A), a UV-VIS detector (L-4250), and an interface (D-6000). The data were collected and analyzed using D7000 HSM software (Merck).

Chromatographic conditions

The separation was performed on a Superspher 100 RP-18 column (endcapped, 4 μm particle size, 125 mm $\times$ 4 mm, Merck) and on a set of 3 Chromolith Performance RP-18e columns (100 $\times$ 4.6 mm, Merck). The mobile phase was prepared according to the method described in the European Pharmacopoeia 2005, by mixing 1.15 g of sodium lauryl sulfate, 10 mL of a mixture of 1 volume of sulfuric acid and 9 volumes of water, 20 mL of 17 g/L solution of tetrabutylammonium dihydrogen phosphate, 370 mL of water and 600 mL of acetonitrile. The pH of the finally resulting solution was adjusted to 3.3 using diluted sodium hydroxide solution. The mobile phase was degassed by sonication before use. The flow rate was 1 mL/min on the conventional column, while different flow rates (1 to 9 mL/min) were applied on the monolithic column. The injection volume was 20 μL and the detection wavelength was 292 nm. All separations were performed at ambient temperature.

Preparation of standards

The primary stock solution of propranolol hydrochloride was prepared in the mobile phase to obtain solutions of known concentrations to be used for the standard preparation and the assay purposes in the range 0.002-1 mg/mL.

Preparation of degradation product

The 2 main degradation products of propranolol hydrochloride are 3-(1-naphthyloxy) propane-1,2-diol and 4-isopropyl-1,7-bis (1-naphthyloxy)-4-azaheptane-2,6-diol. They were generated by basic hydrolysis by the addition of 1 mL of 0.1 M NaOH to a 5 mL solution of 1 mg/mL propranolol hydrochloride in a 25 mL volumetric flask. The solution was left for 20 min to allow hydrolysis. Then 1 mL of 0.1 M HCl was added to neutralize the solution. The volume of the finally resulting solution was completed to 25 mL with mobile phase.

Addition of excipient solution

Inactive ingredients for propranolol hydrochloride tablets were prepared containing the following substances specified as a percentage of tablet weight: propranolol hydrochloride 26.7% w/w, lactose monohydrate 51.3% w/w, microcrystalline cellulose 20% w/w, and magnesium stearate 2% w/w[16].

Results

Method validation

The method used based on the European Pharmacopoeia, but some parameters were changed, including the flow rate and the properties of the octadecylsilyl silica column used. A conventional Superspher 100 RP-18 column (endcapped, 4 μm particle size, 125 mm $\times$ 4 mm, Merck) was used with a flow rate of 1 mL/min, instead of a 5 μm particle size, 200 mm $\times$ 5 mm octadecylsilyl silica column at a flow rate of 1.8 mL/min as specified in Ph. Eur. 2005. The separation was accomplished within 13 min. Propranolol was well separated from its 2 degradation products. The method on the conventional column was found to be successfully transferable to the monolithic columns without modification. It has been demonstrated that the same elution order of the mixture used (propranolol and its 2 degradation products) was obtained on conventional and monolithic columns. This showed that the selectivity of the 2 column types is almost identical. Representative chromatograms for propranolol hydrochloride and its 2 degradation products on conventional and monolithic columns are shown in Figure 2. To ensure assay precision, within day repeatability (n = 5) and between day repeatability (n = 5) were assessed at 3 concentration levels for the conventional (Superspher) as well as a set of 3 monolithic (Chromolith Performance) columns. The 3 tested monolithic columns originated from 3 different batches in a package referred to commercially as a Validation Kit. The results are summarized in Table 1. Monolithic column to column reproducibility was also measured. For the 4 mL/min flow rate selected for precision studies, the within day RSDs % equaled 0.66% for retention time and ranged from 0.38% to 0.92% for peak area. The between days RSD % ranged from 0.52% to 0.72% for retention time and from 0.71% to 1.00% for peak area. The linearity of calibration curves (peak area vs. concentration) for propranolol hydrochloride in the mobile phase were checked over the concentration range 0.002-1.000 mg/mL. The residual plot did not show any trend, correlation coefficients were about 0.9988 and 0.9992 using conventional and monolithic columns, respectively. The limit of detection (LOD, S/N = 3) and an estimate for the limit of quantitation (LOQ, S/N = 10) on monolithic columns at flow rates of 1 mL/min as well as 4 mL/min were 0.012 μg/mL and 0.04 μg/mL, respectively, compared to 0.061 μg/mL and 0.2 μg/mL on the conventional C18 column. The accuracy of the method was tested by determination

of recovery using the inactive ingredient used in the propranolol hydrochloride tablet formulation. The recovery results on both conventional and monolithic columns are summarized in Table 2.

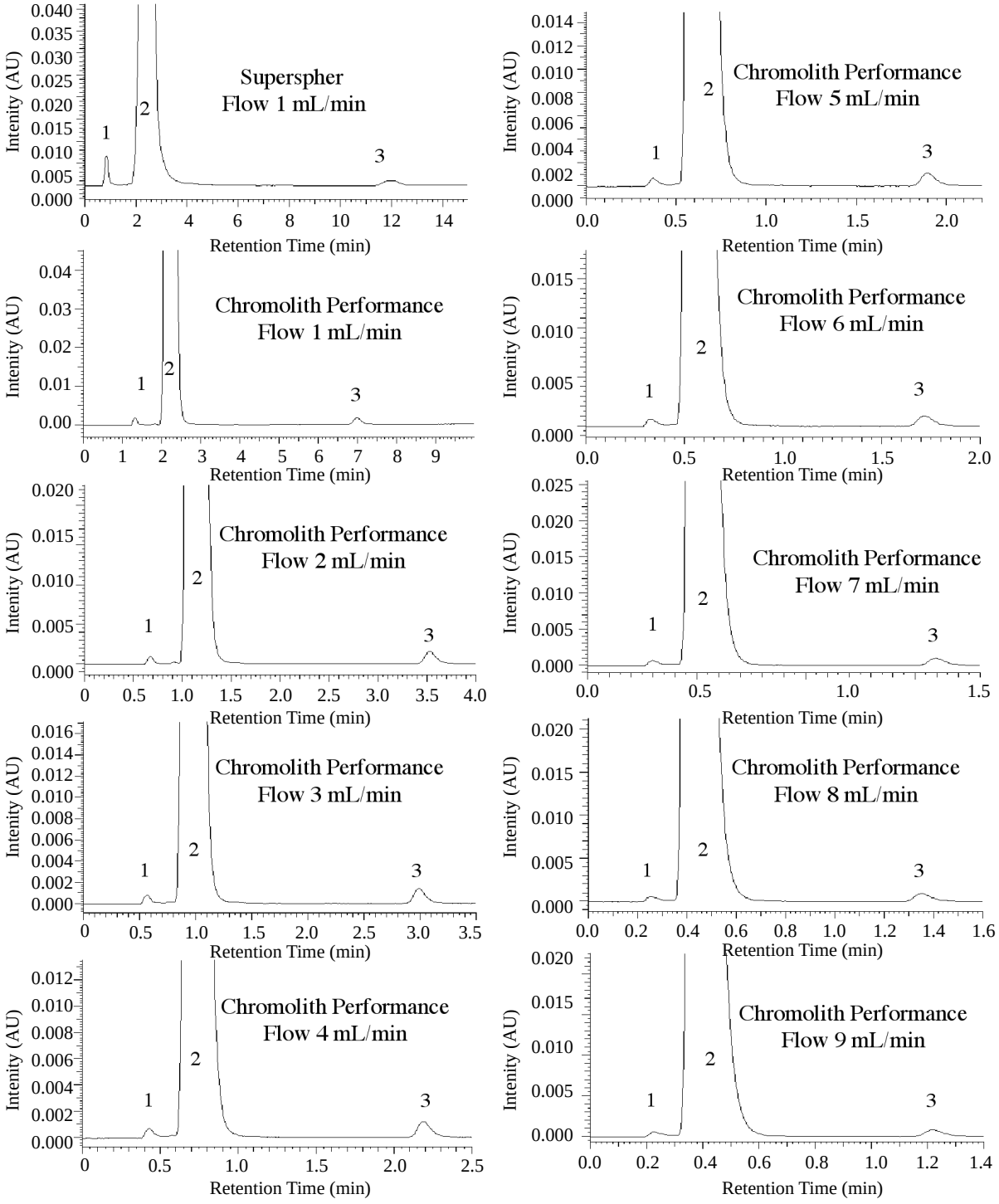


Figure 2. Representative chromatograms for propranolol hydrochloride (peak 2) and its 2 degradation products a & b (peaks 1 & 3, respectively) on conventional (Superspher RP-18) and on monolithic (Chromolith Performance RP-18) columns. Mobile phase consists of buffer pH 3.3: acetonitrile (40:60, v/v).

Table 1. Precision of propranolol on conventional and monolithic columns over the concentration range 0.002-1 mg/mL, using n = 5 for both within day and between days repeatabilities (results on monolithic columns include precision at flow rates of 1 and 4 mL/min).

Column type and No.		Within day repeatability RSD % of AUC			Within day repeatability RSD % of t_g	Between day repeatability RSD % of t_R			Between day repeatability RSD % of t_R
		0.002 mg/mL	0.5 mg/mL	1 mg/mL	(n = 15)	0.002 mg/mL	0.5 mg/mL	1 mg/mL	(n = 15)
Superspher 100 RP-18 column		1.27 %	0.70 %	0.69%	0.76%	1.17%	1.01%	0.97%	0.85%
Chromolith Batch No. Um 1042 Performance RP-18e Rod No. 1042/020	1mL/min	0.78%	0.66%	0.43%	0.42%	0.96%	0.96%	0.81%	0.79%
	4mL/min	0.92%	0.61%	0.57%	0.66%	0.99%	0.87%	0.90%	0.72%
Chromolith Batch No. Um 1043 Performance RP-18e Rod No. 1043/041	1mL/min	0.88%	0.86%	0.61%	0.56%	0.94%	0.85%	0.70%	0.82%
	4mL/min	0.91%	0.96%	0.60%	0.66%	0.95%	1.00%	0.89%	0.52%
Chromolith Batch No. Um 1045 Performance RP-18e Rod No. 1045/036	1mL/min	0.79%	0.61%	0.58%	0.66%	0.89%	0.96%	0.74%	0.90%
	4mL/min	0.92%	0.56%	0.38%	0.66%	0.96%	0.68%	0.71%	0.71%

Table 2. List of recovery results of propranolol hydrochloride from tablet excipient at 3 concentration levels.

Column type	Theoretical value (mg/mL)	Mean recovery* (mg/mL)	Recovery %	RSD %
Superspher RP-18	0.02	0.0197	98.95 %	1.97 %
	0.04	0.0395	98.00 %	1.26 %
	0.08	0.0794	99.27 %	0.91%
Chromolith Performance RP-18	0.02	0.0198	99.00 %	1.91 %
	0.04	0.0398	99.50 %	1.23 %
	0.08	0.0796	99.60 %	1.00%

* Mean value of 10 determinations.

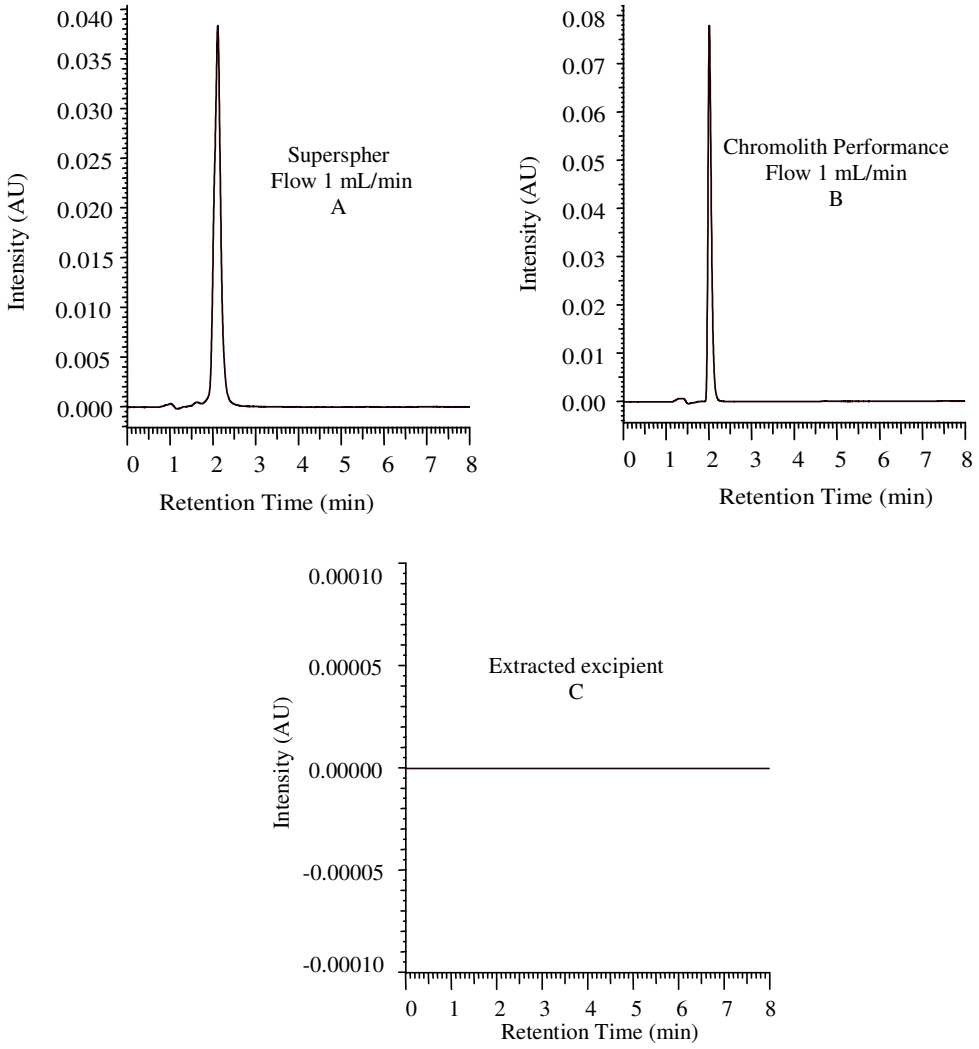


Figure 3. Representative chromatograms for extracted propranolol hydrochloride from tablet excipient on conventional and monolithic columns at a flow rate of 1 mL/min.

The specificity of the method was examined by observing if there was any interference by the inactive ingredients of propranolol hydrochloride tablet excipient. The HPLC chromatograms recorded for propranolol hydrochloride's inactive ingredients showed no peaks at the retention times of propranolol

hydrochloride and its degradation products. Figure 3 shows representative chromatograms for extracted propranolol on the conventional column (chromatogram 3A), on the monolithic column (chromatogram 3B) and for drug-related excipients (chromatogram 3C). The specificity was also demonstrated by the fact that the degradation products obtained by induced degradation of propranolol hydrochloride sample were well separated from it.

Performance parameters

Peak performance parameters were calculated according to fundamental equations (Table 3). Compared to the traditional particulate column, the total run time was shorter in the case of the monolithic columns. This reduces the time consumption for routine series of analyses. Peak resolution was satisfactory and comparable between propranolol and its degradation product (a) using conventional and monolithic columns. Applying higher flow rates on monolithic columns decreases the resolution, but it is still convenient. Peak symmetry is important for precise peak integration and thus for quantitative information. The peak symmetry is better in the case of monolithic columns compared to the conventional column. As expected, a flat curve was obtained by plotting plate height against the flow rates, indicating that monolithic columns can operate at high flow rate with only a small decrease in efficiency. The high permeability of the monolithic columns was evidenced

Table 3. Performance parameters for propranolol on conventional and monolithic columns.

Column type	Theoretical plate N (Plate per column for propranolol hydrochloride)	Asymmetry factor for propranolol peak	Resolution (Rs) propranolol impurity A/propranolol
Conventional C18 (flow rate 1 mL/min)	379	1.4	4.39
Monolithic C18 (flow rate 1 mL/min)	1120	1.1	4.40
Monolithic C18 (flow rate 2 mL/min)	980	1.1	4.18
Monolithic C18 (flow rate 3 mL/min)	925	1.1	3.26
Monolithic C18 (flow rate 4 mL/min)	867	1.2	2.80
Monolithic C18 (flow rate 5 mL/min)	858	1.1	2.00
Monolithic C18 (flow rate 6 mL/min)	792	1.1	1.94
Monolithic C18 (flow rate 7 mL/min)	769	1.1	1.80
Monolithic C18 (flow rate 8 mL/min)	757	1.2	1.66
Monolithic C18 (flow rate 9 mL/min)	694	1.1	1.58

*The following equations were used to calculate the above-mentioned chromatographic parameters: ($N = 16\ (t_R/w)^2$, asymmetry factor (AF) = B/A at 10% of peak height (A & B are the two half width at each side of the peak center) and Resolution $Rs = 2(t_{R2}\text{-}t_{R1})/(w_2 + w_1)$

by a total system back pressure of about 133 bars at a flow rate of 9 mL/min. In the conventional column more time was required to re-equilibrate (about 30 min) or to wash the stationary phase. In contrast, the time required to re-equilibrate (about 5 min) or wash the monolithic column was markedly shorter, probably due to the rigidity of this type of column.

Discussion

Both types of column were able to separate propranolol from its 2 degradation products; however, the data presented in this article show explicit advantages of monolithic C18 columns compared to the conventional C_{18} column with 4 μm particles. Monolithic columns were found to perform the separation with a shorter run time, sufficient resolution and better peak symmetry as compared to the conventional column. The analysis on monolithic columns is applicable under the same chromatographic conditions specified for the conventional column. Both within day and between days variations were also slightly better on monolithic columns than on the conventional column, possibility due to the better peak shape and reduced baseline noise. When higher flow rates were applied on monolithic columns there was some albeit minor slow loss in resolution. The separation efficiency of monolithic columns was found to decrease slowly when the flow rate was increased, in contrast to the traditional particulate column. This could be explained by the improved mass transfer of monolithic over conventional columns at high flow rates. A flow rate of 4 mL/min was selected for precision studies on monolithic columns, as it provides the smallest analysis time, keeping resolution values higher than 2 (Rs $>$ 2). The total analysis time was reduced to about 20% at a flow rate of 4 mL/min using monolithic columns. At a flow rate of 9 mL/min the 3 peaks were eluted within less than 1.4 min. The same mobile phase was applied on conventional and monolithic columns, to enable the direct comparison of the 2 columns' performances and to see if a chromatographic method could be transferred from conventional to monolithic columns without further modification. The high permeability of the monolithic columns was evidenced by the fact that a flow rate of 9 mL/min generated a total system back pressure of only 108 bars. In comparison, the conventional column packed with 4 μm particles reached a back pressure of about 379 bars when it was operated at a flow-rate of 4.5 mL/min using the same mobile phase. At a flow rate of 1 mL/min back pressure is about 6-fold smaller on the monolithic than on the conventional particle-packed column. The lower limits of detection and quantitation obtained by monolithic columns are partly due to the lower background noise obtained with monolithic columns. The method is applicable for the rapid quantitation of propranolol and its degradation product. Furthermore, the method could also be useful for stability testing of propranolol hydrochloride formulations. A clear advantage of the monolithic columns is the ability of using high flow rates regardless of back pressure. Monolithic columns have been shown as an excellent alternative to conventional silica based columns.

Acknowledgments

We are grateful to Simone Schröder for carefully reviewing the manuscript.

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