PERFORMANCE EVALUATION OF DIFFERENT HPLC COLUMNS IN SILDENAFIL AND TADALAFIL ANALYSIS

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INTRODUCTION

Sildenafil and tadalafil are oral drugs used to treat male sexual function problems (impotence or erectile dysfunction) by blocking an enzyme 5-phosphodiesterase in the body. However, there is no analytical method for determination of these two active compounds in pharmaceutical preparations in the current European and US Pharmacopoeia. The aim of this study was to evaluate performance of the various HPLC columns in sildenafil and tadalafil analysis using validated HPLC method.

MATERIALS AND METHODS

The following columns were compared: LiChrospher® 100 RP-18 (250 x 4 mm i.d., 5 μm); Hypersil BDS-C18 (125 x 4 mm i.d., 5 μm) and Chromolith® Performance RP-18e (100 x 4.6 mm i.d., monolithic rod). The performance evaluation was done by comparison of the following parameters: resolution (*R*s), back-pressure (Δ*P*, bar), and theoretical plate height (*ΔH*, μm) in correlation with flow-rate (*u*, mL/min).

HPLC analyses were performed using a Schimadzu LC-2010 chromatographic system (Schimadzu, Kyoto, Japan) consisting of a LC-20AT Prominence liquid chromatograph pump with DGU-20A5 Prominence degasser, a SPD-M20A Prominence Diode Array Detector, RF 10AXI fluorescence detector and a SIL-20 AC Prominence auto sampler. Data analyses were done using Class VP 7.3 Software. The mobile phase consisted of a phosphate buffer (20 mM, pH 2.8)-acetonitrile (71:29, *V/V*) at controlled temperature (25°C) and autosamler temperature at 4 °C. Detection of sildenafil and tadalafil was carried out at 285 nm.

RESULTS AND DISCUSSION

Chromatographic peak resolution data (*R*s) obtained are acceptable for all three tested columns with values higher then the limit given in Ph.Eur. (>1.5) (Fig. 1). The best peak resolution data showed the longest column, as it was expected.

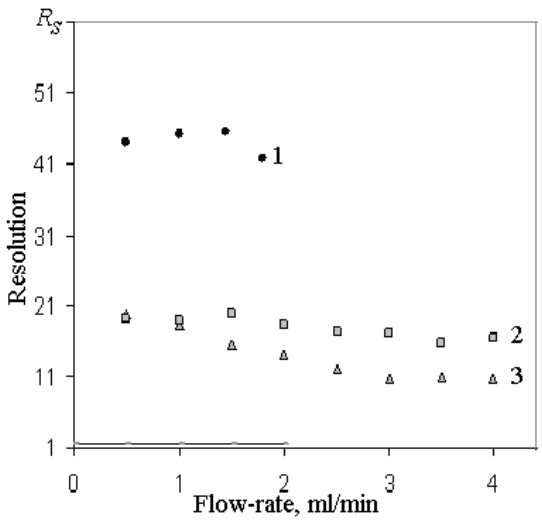

Fig. 1: Correlation between flow-rate and resolution: LiChrospher® 100 RP-18 (1); Hypersil BDS-C18 (2) and Chromolith® Performance RP-18e (3).

There is a significant difference between column back-pressure using different flow-rate. Thus, the longest column was tested only at flow-rate up to 2 ml/min because its high back-pressure induced by increasing flow-rate. The other two columns were tested up to flow-rate of 4 ml/min. The monolithic rod column generates fourfold lower back-pressure in comparison with the longest column, and nearly twice lower back-pressure then the other column.

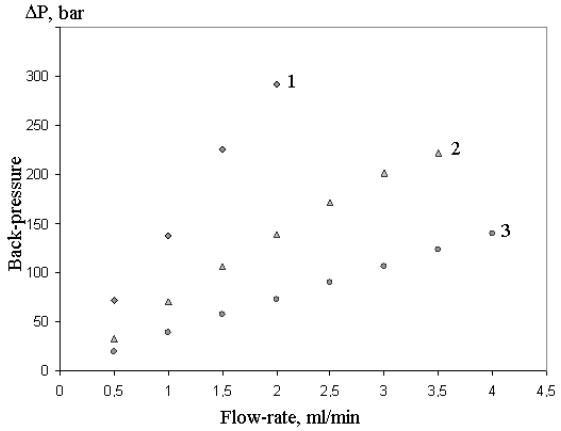

Fig. 2: Correlation between flow-rate and back-pressure: LiChrospher® 100 RP-18 (1); Hypersil BDS-C18 (2) and Chromolith® Performance RP-18e (3).

The efficiency of the columns is presented by the van Deemter plots. According to the results, the most efficient column is the longest column packed with particles, but the flow-rate which might be used is limited at maximum 2 ml/min. The shorter column packed with particles showed the worst efficiency in comparison with other tested columns. This column has acceptable efficiency only up to flow-rate of 1 ml/min. With increasing flow-rate its efficiency dramatically decreases. The van Deemter plot of the monolithic rod column demonstrates clearly that separation efficiency does not decrease significantly when the flow-rate is increased, as it is the case with particulate columns. Therefore it is possible to operate with this type of columns at higher flow-rate without loss of peak resolution. The same conclusion for efficiency of the columns is obtained for the both tested compounds, but the results obtained for tadalafil are better in comparison with those for sildenafil.

CONCLUSIONS

According to all experimental results obtained, the monolithic rod column is a column of choice for tadalafil and sildenafil analysis. Using this column means shorter analysis time (for factor 3.6) in comparison with the longest column. Additionally, it is important to be mentioned that decreased consumption of organic solvent considerably reduces the laboratory expenses.

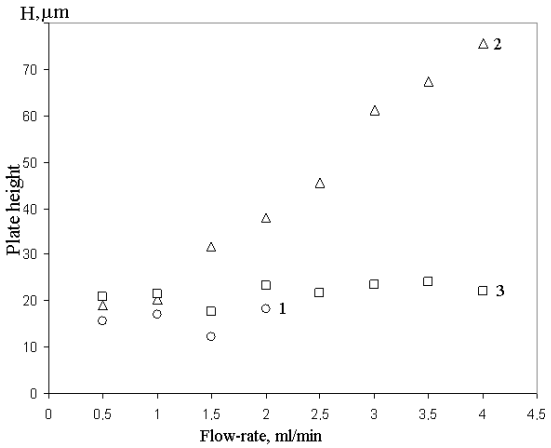

Fig. 3: Correlation between flow-rate and plate height obtained for sildenafil: LiChrospher® 100 RP-18 (1); Hypersil BDS-C18 (2) and Chromolith® Performance RP-18e (3).

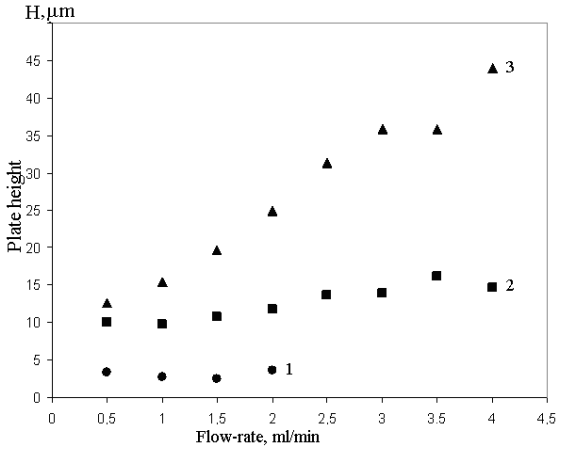

Fig. 4: Correlation between flow-rate and plate height obtained for tadalafil: LiChrospher® 100 RP-18 (1); Hypersil BDS-C18 (2) and Chromolith® Performance RP-18e (3).

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