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Short communication

Evaluation of chromatographic conditions for simultaneously determination of Emtricitabine and Tenofovir

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Introduction

The nucleoside analogue reverse transcriptase inhibitor emtricitabine and the nucleotide analogue RTI tenofovir disoproxil fumarate are antiviral drugs. They have each shown antiviral activity against a number of HIV clinical isolates and cell lines. A fixed-dose combination of two antiretroviral drugs (tenofovir and emtricitabine) used for the treatment of HIV is already present on the European market (Dando and Wagstaff, 2004)

However, monographs of emtricitabine and tenofovir are not included in the actual European Pharmacopoeia (Ph. Eur. 10th Edition). There is no official method for quality control of pharmaceutical dosage forms containing both emtricitabineand tenofovir. Therefore, reliable HPLC methods for simultaneous analysis of emtricitabine and tenofovir are needed for quality control of the approved fixed-dose combination of two the antiretroviral drugs.

The mostly used RP-HPLC columns in the quality control of the pharmaceuticals are those packed with silica particles with irregular or spherical shape, or could be monolith rods. On their surface there is octa-decyl-silyl (ODS, C18) reverse-phase coating. However, the chromatography performance obtained from defferent brand of the RP-HPLC columns differs significantly between each other, due to the quality of the column silica. The content of the metal ions residues in the silica could significantly influence the column performance (Bhavsar et al., 2012).

Few UV, RP-HPLC, high performance thin layer chromatography (HPTLC), and liquid chromatography with tandem mass spectrometry (LC/MS/MS) methods have been published for simultaneous estimation of emtricitabine and tenofovirdisoproxil fumarate in pharmaceutical formulation. However, most of them require sofisticated equipment and time consuming sample preparation (Bhavsar et al., 2012).

Therefore, the aim of this study was to optimize and to propose chromatography conditions for simultaneous identification and determination of emtricitabine and tenofovir active compounds in pharmaceutical dosage forms suitable for routine analysis in the quality control laboratories.

Materials and methods

Materials and reagents

HPLC analyses were performed using an Agilent Technologies chromatographic system (Hewlett Packard, Avondale, USA) consisting of a binary pump with DAD detector and auto sampler controlled by Agilent Technologies HPLC 1100 software.

Chromatographic separation was achieved on following analytical HPLC columns: Hypersil BDS C18 (125 mm x 4 mm); Zorbax SB C18 (250 mm x 4.6 mm); Lichrospher 100 C18 (250 mm x 4 mm); Purospher C18 endcapped (150 mm x 4.6 mm); Purospher C18 endcapped (250 mm x 4.6 mm), X-Select HBB C18 (250

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mm x 4.6 mm), all with particle size 5 µm and Chromolith 18e performance (100 x 4.6), rod.

The mobile phase was composed of mixture of acetonitrile and water acidified with o-phosphoric acid (pH 2.6) in ratio 30/70 (V/V), with flow rates ranging from 1 to 3 mL/min, filtered through 0.45 µm nylon filter. The column temperature was set at 30 °C, injection volume 10 µL, with operating wavelengths at 260 nm and 280 nm.

Preparation of standard solutions

The stock solutions of standards were prepared with quantities of 16.35 mg emtricitabine and 24.53 mg tenofovirdisopronil succinate accurately weighted, transferredin separate volumetric flasks with volume of 25 mL and dissolved in a mixture of acetonitrile and water acidified with o-phosphoric acid (pH 2.6) in ratio 40/60 (*V/V*). We mixed the obtained solutions at an ultrasonic bath for 5 min. Then, for preparing the test solution the volumes of 3 mL from each of two stock solutions were mixed in 10 mL volumetric flask and filled up to the volume with the same solvent.

Results and discussion

In this study we used seven different ODS columns and we evaluate their efficiency in emtricitabine and tenofovir analysis.

Testing for identification, specificity, selectivity, resolution and suitability was according to requirements in the ICH Q2(R1) Guideline (ICH, 2019).

UV spectra scanned in mobile phase during the chromatographic analysis showed absorption maximum at 220 nm, 237 nm and 284 nm for emtricitabine and at 210 nm and 260 nm for tenofovir.

From the evaluated HPLC columns only two did not separatethe two analysed chromatographic peaks, at the applied chromatographic conditions: (Hypersil BDS C18 (125 mm x 4 mm) and Zorbax SB C18 (250 mm x 4.6 mm)). All other columns showed satisfactory resolution, over 4.3.

The best separation factor (ά) values are obtained for Purospher C18 endcapped (150 mm x 4.6 mm) and Purospher C18 endcapped (250 mm x 4.6 mm) (4.3 and 6.1, respectively).

The highest number of theoretical plates (N) were also estimated for the Purospher C18 endcapped (150 mm x 4.6 mm) columns and Purospher C18 endcapped (250 mm x 4.6 mm) columns (11677 and 16812 for the shorter and 20736 and 13114 for the longer column, respectively). We also obtained the best values for the height of theorethical plates withPurospher C18 endcapped columns.

The best peak symmetry was achieved on columns: Lichrospher 100 C18 (250 mm x 4 mm) and Purospher C18 endcapped (150 mm x 4.6 mm) at flow-rate of 1 mL/min and on Chromolith 18e performance (100 x 4.6), rod, at flow-rate 3 mL/min. These three columns showed retention times for emtricitabine (2.03; 1.81; 0.52 min) and for tenofovir (2.62; 5.77; 1.17 min) consequently.

The analysis run-timewith the studied columns ranged from6 min to 13 min maximum, which is optimal run-time for the routine control in the pharmaceutical laboratories.

Conclusion

The emtricitabine and tenofovir can be successively separated withRP–HPLC octadecylsylil columns.

Our experimental results showed that the optimal chromatographic conditions for simultaneous HPLC analysis of emtricitabine and tenofovir are achieved with mobile phase composed of 30% acetonitrile and 70%, water acidified with o-phosphoric acid at pH 2.6, at flow-rate 1 mL/min, column oven set at 30ºC, with specific UV detection at 260 nm for tenofovir and at 284 nm for emtricitabine.

Optimal results were obtained withLichrospher 100 C18 (250 mm x 4 mm) and Purospher C18 endcapped (150 mm x 4.6 mm) columns.

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