IDENTIFICATION OF COUNTERFEIT MEDICINES FOR ERECTILE DYSFUNCTION BY VALIDATED RP-HPLC METHOD

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The aim of the study was to develop a specific, sensitive, simple, and rapid RP-HPLC method with UV detection for identification of counterfeit medicines for erectile dysfunction: containing vardenafil, sildenafil, and tadalafil. HPLC analysis was 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, and a SIL-20 AC Prominence auto sampler. Data analyses were done using Class VP 7.3 Software. The elution was carried out on a column Chromolith® Performance RP-18e (50 x 4.6 mm i.d., monolithic rod), with a mobile phase consisted of acetonitrile and 0.02 mol L^{-1} phosphate buffer (pH = 2.8) in a ratio of 29:71, (*V/V*), at flow rate of 0.6 mL min^{-1}, at controlled column temperature (40°C) and autosamler temperature at 4°C. Detection of vardenafil, sildenafil, and tadalafil was carried out with DAD detector at a wavelength of 285 nm. The injection volume was 20 µl. The samples included medicines for erectile dysfunction (sildenafil 50 mg tablets, tadalafil 20 mg tablets, vardenafil 10 mg tablets) and were submitted by the state regulatory authority, Bureau of the medicines. The method was fully validated according to the ICH (International Conference on Harmonization) guidelines by the determination of linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). Selectivity of the method was proved with the chromatographic peak resolution obtained between the peaks of vardenafil, sildenafil, and tadalafil and the characteristic UV spectra. Linearity of the method was tested in the range of 2.5 – 100 µg mL^{-1} for all tested substances. Experimental data showed high level of linearity with the values of the correlation coefficients (R^2 = 0.9991, R^2 = 0.9995, R^2 = 1.0 for for vardenafil, sildenafil, and tadalafil, respectively). The LOD and LOQ for vardanafil were 4.86 ng mL^{-1} and 14.72 ng mL^{-1}, respectively; the LOD and LOQ for sildenafil were 3.75 ng mL^{-1} and 11.37 ng mL^{-1}, respectively; while the LOD and LOQ for tadalafil were 0.56 ng mL^{-1} and 1.69 ng mL^{-1}, respectively. The accuracy of the method was demonstrated by the values obtained from the recovery experiments (100.06%, 99.57%, and 99.53% for vardenafil, sildenafil, and tadalafil, respectively). The proposed HPLC method allows a simple, accurate, precise and rapid identification and determination of vardenafil, sildenafil, and tadalafil in potentially counterfeit products.

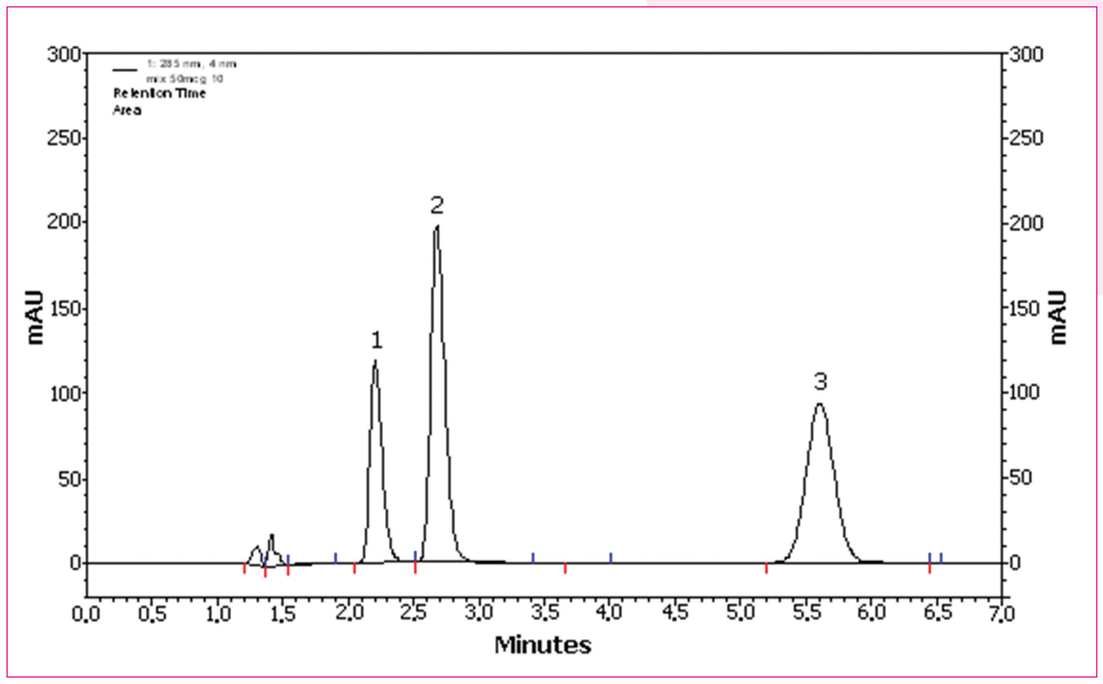

Figure 1. Spectra of standard solutions of: vardenafil, sildenafil and tadalafil (a) and a chromatogram of mix standard solution of: vardenafil, sildenafil and tadalafil (b)

References

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