Validated HPLC method for determination of sildenafil in pharmaceutical dosage forms

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INTRODUCTION

Sildenafil is oral drug used primarily to treat male sexual function problems (impotence or erectile dysfunction) since becoming available in 1998. It is a potent and selective inhibitor of cGMP specific phosphodiesterase type 5 (PDE5) in the corpus cavernosum, where PDE5 is responsible for degradation of cGMP. Sildenafil has a peripheral site of action on erections. This substance has no direct relaxant effect on isolated human corpus cavernosum but potently enhances the relaxant effect of NO on this tissue.

However, there is no analytical method for determination of this active compound in pharmaceutical preparations in the current European and US Pharmacopoeia.

The aim of this study was to develop and validate HPLC method for sildenafil analysis in pharmaceutical dosage forms.

MATERIALS AND METHODS

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, RF 10AXI fluorescence 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 Hypersil BDS-C18 (125 x 4 mm i.d., 5 μm), mobile phase consisted of phosphate buffer (20 mM, pH 2.8)-acetonitrile (71:29, *V/V*), flow rate 1.5 mL min^{-1}, at controlled temperature (25°C) and autosamler temperature at 4°C. Detection of sildenafil was carried out at 285 nm.

Commercially available, film-coated tablets, containing 50 mg sildenafil citrate, were used in this study.

CONCLUSIONS

The results of the validation demonstrated that the analytical procedure is accurate, precise and reproducible for sildenafil analysis in pharmaceutical dosage forms.

The proposed method was successfully applied for determination of sildenafil in pharmaceutical formulations. This analytical procedure is relatively inexpensive and simple and is particularly suitable for routine analyses when tandem mass spectrometric detection is not available. Additionally, it is important to mention that decreased consumption of organic solvent considerably reduces the laboratory expenses.

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RESULTS AND DISCUSSION

The method was fully validated according to the ICH (International Conference on Harmonization) guidelines by determination of linearity, precision, accuracy, limit of detection and limit of quantification. Selectivity of the method was proved with the chromatographic peak resolution obtained between sildenafil and tadalafil (Rs = 10,5)(Fig. 1) and the characteristic UV-spectrum (Fig. 3 (B)).

Linearity of the method was tested in range: 2 – 100 μg mL^{-1} (Fig. 2). Experimental data showed high level of linearity proved with the value of the correlation coefficient (R^2 = 0.9994).

LOD and LOQ of the method were tested in the range: 20 – 200 ng mL^{-1} sildenafil. The limits of the method are 0.23 ng and 0.68 ng for LOD and LOQ, respectively (or 9.2 ng mL^{-1} and 27.2 ng mL^{-1} for LOD and LOQ, respectively, by injecting 25 μL on a column).

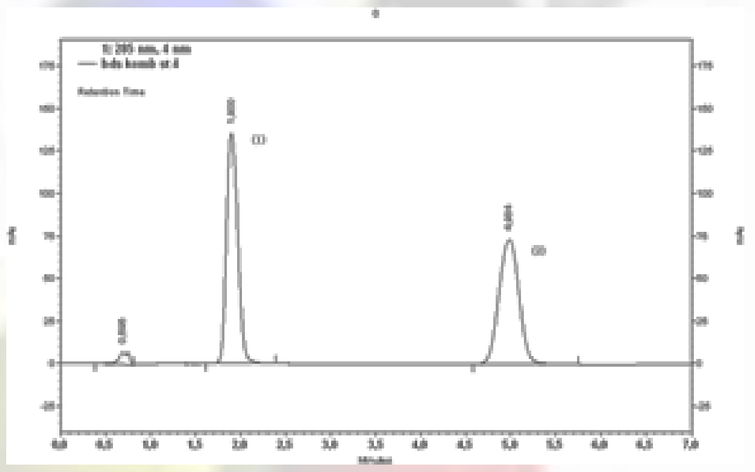

Fig. 1: Characteristic chromatogram obtained with the mixed standard solution using proposed HPLC-method: sildenafil (1), tadalafil (2).

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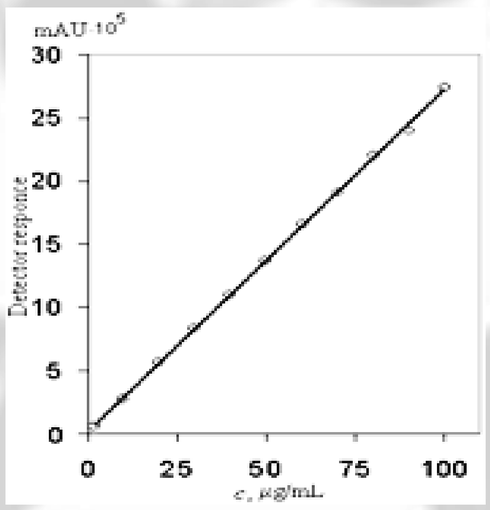

Fig. 2. A typical sildenafil calibration line, with regression line (Abs = 27066 conc. + 14033, and R2 = 0,9994)

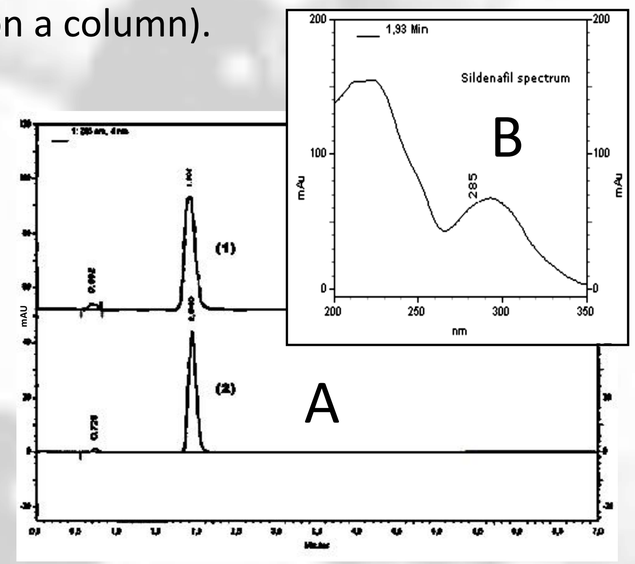

Fig. 3: (A) Characteristic chromatogram obtained using proposed HPLC-method: sildenafil (1) in refernce solution, sildenafil (2) in test solution (B) UV Spectrum

Table 1. Accuracy of the analytical method

	Film-coated tablets containing 50 mg sildenafil as sildenafil citrate		
	Added amount* (μg/ml)	Found amount* (μg/ml)	Recovery (%)
	18,50	18,45	99,74
	24,50	24,13	98,49
	30,50	30,77	100,88
$\overline{X}$ ± SD			99,70 ± 1,20
RSD (%)			1,20

* Average of three determination