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Separation and Determination of Synthetic Impurities of Sildenafil (Viagra) by Reversed-Phase High-Performance Liquid Chromatography

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A simple and rapid high-performance liquid chromatographic method for the separation and determination of process-related impurities of sildenafil was developed. The separation was achieved on a reversed-phase C_{18} column using acetonitrile–0.05 M potassium dihydrogen orthophosphate (70:30 v/v) as a mobile solvent at a flow rate of 1.0 ml/min and UV detection at 230 nm. The method was used not only for quality assurance, but also for monitoring the chemical reactions during the synthesis of sildenafil. It was found to be specific, precise and reliable for the determination of all process-related impurities of sildenafil in bulk drugs and formulations.

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Introduction

Sildenafil,1-{[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]}-4-methylpiperazine (SLD), popularly known as Viagra, is a novel oral agent for the treatment of penile erectile dysfuntion, which consists of an inability to achieve or maintain a hard, erect penis sufficient for sexual intercourse.[1,2] It is an active inhibitor of the type V-cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase on penile erectile activity, and causes cGMP to accumulate corpus cavernosum.[3-6] The clinical trails involving pharmacokinetic, efficacy and safety evaluations have revealed that SLD is an effective and well-tolerated drug in the treatment of male erectile dysfuntion.[7] Its synthesis[8] involves several steps, in which 4-amino 1-methyl-3-propylpyrazole-5-carboxamide (AMP) is initially obtained from 1-methyl-4-nitro-3-propylpyrazole-5-carboxamide (MNC) by reduction with $SnCl_2$ under refluxed conditions. It is then condensed with 2-ethoxybenzoyl chloride and oxidized with H_2O_2 to yield 5-(2-ethoxyphenyl)-1-methyl-3-propyl-6,7-dihydro-1*H*-pyrazolo-[4,3-d]pyrimidin-7-one (EMP), which is then sulfonated with chlorosulfonic acid to give 5-[5-(chlorosulfonyl)-2-ethoxyphenyl]-1-methyl-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-d]-pyrimidon-7-one (CMP). In the last step, CMP is reacted with methyl piperazine to produce sildenafil (SLD), which is generally converted into citrate and used for treatment without further purification.[9] Since it is intended for oral consumption, its purity and safety can be thoroughly ensured before using it in different formulations. Its quality depends not only on the adopted procedures, but also on the synthetic precursors, side reaction products, unreacted raw materials, and intermediates, since they may possess unwanted toxicological effects due to which the benefit from administration of SLD may be outweighed. Therefore, a close monitoring of related substances is of great importance for controlling the quality of SLD in the final products. A thorough literature search has indicated that the voltammetric behavior of SLD using square-wave and adsorptive stripping techniques in pharmaceutical preparations was studied.[10] A flow injection analysis (FIA) method using UV detection for the determination of SLD in pharmaceutical preparations was reported.[11] However, these methods were found not to be suitable for differentiating the related substances from SLD due to a lack of selectivity. Thus, HPLC is the technique of choice for the separation and determination of related impurities in pharmaceutical preparations. A few methods based on HPLC were reported for the determination of sildenafil citrate in biological and pharmaceutical products. A reversed-phase HPLC method using acetonitrile–phosphate buffer–water (28:4:68 v/v/v) with detection at 230 nm was utilized for the simultaneous determination of sildenafil and its metabolite (UK-103, 320) using the automated sequential trace enrichment of dialysates.[12] This method was found to be selective and precise, but not stable, thus indicating that most of the impurities have short retention times close to that of the solvent front, and not resolved from each other. Segall *et al*. have proposed a reversed-phase HPLC method using 70 mM potassium phosphate monobasic buffer of pH 3.0 containing 100 mM triethylamine:acetonitrile (7:3 v/v) as the mobile phase at 225 nm for the separation and determination of the degradation products of sildenafil citrate formed due to oxidation.[13] Dinesh *et al*. developed another reversed-phase HPLC method for the determination of SLD in pure and pharmaceutical forms using a

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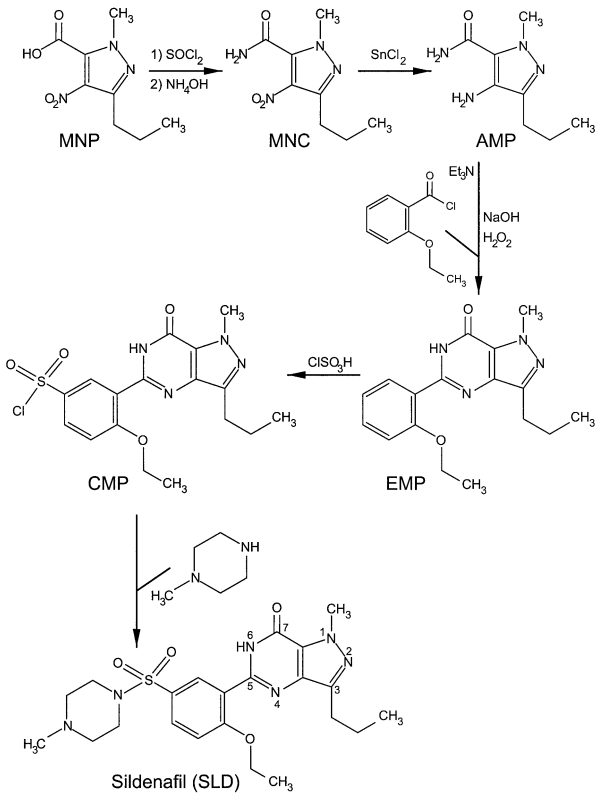

Fig. 1 Reactions involved in the synthesis of sildenafil.

Lichrospher C_{18} column with water–acetonitrile as the mobile phase and UV detection at 245 nm.[14] A reversed-phase HPLC method for the determination of its related substances in commercial formulations and tablets was reported.[15] However, it did not attempt to separate 1-methyl-4-nitro-3-propylpyrazole-5-carboxylic acid (MNP), which is most likely to be present as an impurity in the final products of SLD. In this paper we describe a simple and rapid reversed-phase HPLC method for the separation and determination of small amounts of all probable impurities of SLD, not only for quality assurance, but also to monitor the procedures followed during its synthesis.

Experimental

Materials and reagents

Analytical reagent-grade potassium dihydrogen orthophosphate (E. Merck, Mumbai, India) and HPLC-grade acetonitrile obtained from Qualigens, Mumbai, India was used. Glass-distilled and deionized water (Nanopure, Barnsted, USA) was used throughout the study. Sildenafil and its synthetic impurities were synthesized by D. S. in his laboratory department of chemistry (Dr. Hari Singh Gour University, Sagar, India) and used.

Apparatus

An HPLC system was composed of two LC-10 AT VP pumps, an SPD-M 10A VP diode array detector, an SIL-10AD VP auto injector, a DGU 12 A degasser and an SCL-10 VP system controller (all from Shimadzu, Kyoto, Japan). A reversed-phase C_{18} (YMC, Kyoto, Japan) column (25 cm × 4.6 mm i.d., particle size 5 μm) was used for separation. The chromatographic and integrated data were recorded using an HP-Vectra (Hewlett Packard, Waldron, Germany) computer system.

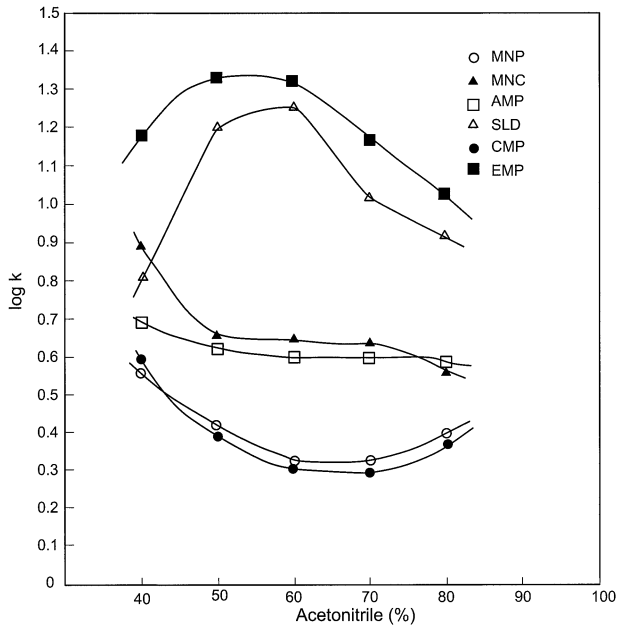

Fig. 2 Effect of the concentration of acetonitrile on the retention of sildenafil and its impurities.

Chromatographic conditions

The mobile phase was acetonitrile–0.05 M potassium dihydrogenphosphate (70:30 v/v); before delivering into the system it was filtered through 0.45 μm PTFE filter and degassed using a vacuum. The analysis was carried out under isocratic conditions using a flow rate of 1.0 ml/min at room temperature (28˚C). Chromatograms were recorded at 230 nm using an SPD-M 10A VP diode array detector.

Analytical procedure

Samples (5 mg) were dissolved in the mobile phase (10 ml) and a 20 μl volume of each sample was injected and chromatographed under the above conditions. Synthetic mixtures containing CMP, MNP, AMP, MNC, SLD, EMP and bulk drugs were analyzed under identical conditions. The amounts of impurities were calculated from their respective peak areas.

Results and Discussion

Figure 1 shows the chemical reactions generally followed in the synthesis of SLD in a bulk drug manufacturing unit. It can be seen from Fig. 1 that there are as many as six compounds, which include the starting materials and intermediates, that could be present as potential impurities in SLD. The present study was aimed at developing a chromatographic system capable of eluting and resolving SLD and its impurities originating from synthesis. In a preliminary experiment, all of these impurities and SLD were subjected to separation by reversed-phase HPLC using a YMC C_{18} column and acetonitrile–water as an eluent. Two compounds *viz*., EMP and SLD were retained on the column when the concentration of acetonitrile was kept below 40%. However, upon increasing its

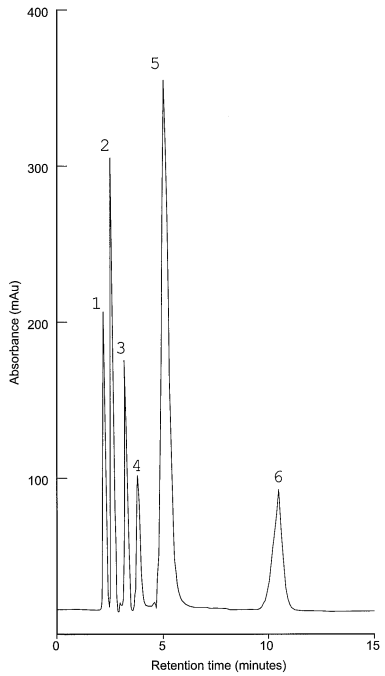

Fig. 3 Typical chromatogram of a synthetic mixture containing (1) CMP (5 μg), (2) MNP (5 μg), (3) AMP (5 μg), (4) MNC (5 μg), (5) SLD (5 μg) and (6) EMP (5 μg).

concentration, these two compounds were eluted, but the separation of the other compounds was found to be effected. This would most probably be due to the adsorption of these two compounds by exposed silinols on C_{18} material of the HPLC column. This behavior of SLD and EMP is reasonable because both compounds have basic functional groups with pK_a values of 8.7 and 8.0 with a weak acidic moiety on the parent compound. In another attempt, the water was replaced by 0.05 M potassium dihydrogenphosphate, and the effect of the concentration of the organic modifier *viz*., acetonitrile upon separation was studied (Fig. 2). When the concentration of acetonitrile was at 70%, all of the impurities and SLD were eluted and separated from one another. The typical chromatogram of a synthetic mixture containing SLD and its impurities is shown in Fig. 3. The peaks were identified by injecting and comparing with the retention times of the individual compounds. It can be seen from Fig. 3 that SLD was well separated from all of the impurities examined in the present study, and that these impurities were also well separated from each other. The specificity of the method was checked by subjecting the bulk drug under UV light at 254 nm and to stress conditions, like 0.1 M HCl, 0.1 M NaOH and 3% H_2O_2 solutions at 60˚C and for 24 h. All of the degraded products were found to be well-separated from SLD, and did not interfere with any of the process impurities, indicating that the method is quite specific. The chromatographic data, including the retention times (t_R), retention factors (k), number of theoretical plates (N), tailing factors (T_f), relative response factors (RRF) and wavelength of absorption maxima (λ_{max}), are given in Table 1.

A synthetic mixture containing small quantities of impurities *viz*., MNP, MNC, AMP, CMP, EMP and SLD, was prepared and chromatographed to check that these quantities were accurately reflected in their peak areas. All of the estimations were carried out thrice and the percentage of error was calculated (Table 2). The precision of the method was

Table 1 Retention and response data for SLD and potential impurities

	t_R/min	RRT[a]	k	N	T_f	RRF[b]	λ_{max}/nm
CMP	2.20	0.42	2.66	2724	0.97	3.17	300
MNP	2.43	0.46	3.05	6889	0.96	4.34	279
AMP	3.16	0.60	4.27	5025	1.07	3.76	235
MNC	3.60	0.68	5.00	6978	0.96	5.32	280
SLD	5.29	1.00	7.82	3818	1.10	1.00	298
EMP	10.38	1.96	16.30	9369	1.18	2.15	233

CMP, 5-[5-(chlorosulfonyl)-2-ethoxyphenyl]-1-methyl-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-d]pyrimidin-7-one; MNP, 1-methyl-4-nitro-3-propylpyrazole-5-carboxylic acid; AMP, 4-amino-1-methyl-3-propylpyrazole-5-carboxamide; MNC, 1-methyl-4-nitro-3-propylpyrazole-5-carboxamide; SLD, sildenafil; EMP, 5-(2-ethoxyphenyl)-1-methyl-3-propyl-6,7-dihydro-1*H*-pyrazolo-[4,3-d]pyrimidin-7-one.
a. RRT: relative retention time.
b. RRF: relative response factor.
n = 6.

Table 2 Recovery data for standard mixtures containing CMP, MNP, AMP, MNC, SLD and EMP

Compound	Taken, %	Found[a]	Error, %
CMP	1.17	1.20 ± 0.03	2.56
MNP	0.80	0.83 ± 0.04	3.75
AMP	0.73	0.71 ± 0.03	2.74
MNC	1.14	1.12 ± 0.04	1.75
SLD	95.40	94.87 ± 1.19	0.55
EMP	0.76	0.74 ± 0.03	2.63

a. Mean ± SD (n = 6).

determined on 10 replicate injections of SLD solution, and reported as the relative standard deviation (RSD), 0.89%. The signal-to-noise ratio was determined to be 4.0 for the detection of impurities as low as 0.02×10^{-9} g. It can be seen from Table 2 that the measured amounts agree well with the actual values; the mean recovery of SLD from authentic samples was found to be 99.75 ± 0.25%. The UV detector was set at 230 nm for both detection and quantification. The wavelength was selected based on observations that the resolution between the chromatographic peaks of SLD and its synthetic impurities were better.

A bulk drug of SLD was spiked with low-level impurities and chromatographed. The chromatogram is shown in Fig. 4. The high-level (75% – 120%) linearity of SLD as well as the low-level (0.05% – 0.5%) linearity data of impurities were determined, and are recorded in Table 3. Good linearity was found between the mass and the integral response for each of the compounds under examination. Table 3 gives the linearity equation, mass range and correlation coefficients for all of the compounds. At 0.001 A.U.F.S., the limit of detection (LOD) of SLD was 6.00×10^{-9} g; the impurities are recorded in Table 3. The robustness of the method was evaluated by a deliberate slight variation of the parameters, such as the mobile-phase composition, strength of potassium dihydrogenphosphate, temperature, flow rate and wavelength of absorption for detection. No significant change was observed in the chromatographic results of SLD and its impurities, even after changing the experimental parameters.

The quality of SLD in bulk drugs was thoroughly checked. A typical chromatogram (T_{0h}) of a bulk drug of SLD is shown in

Table 3 Linear-regression analysis data of SLD and its related impurities

Compound	Mass range/10^{-9} g	Linear regression	r	Limit of detection/10^{-9} g
CMP	0.10 – 1.50	$2.193 \times 10^6x - 534556.22$	0.984	0.04
MNP	0.10 – 0.50	$1.584 \times 10^6x - 4218.47$	0.992	0.02
AMP	0.10 – 0.50	$1.866 \times 10^6x - 9327.54$	0.997	0.04
MNC	0.10 – 2.50	$3.030 \times 10^6x + 6567.43$	0.986	0.03
SLD	90.5 – 99.9	$6.944 \times 10^6x - 24153.61$	0.995	6.00
EMP	0.10 – 0.50	$4.342 \times 10^6x + 5355.79$	0.991	0.04

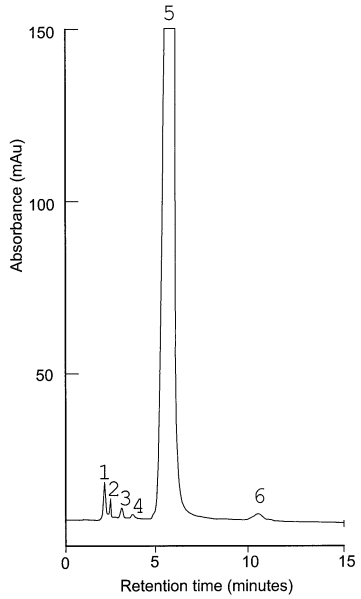

Fig. 4 Typical chromatogram of (5) SLD (20 μg) spiked with low-level impurities containing (1) CMP (0.5 μg), (2) MNP (0.5 μg), (3) AMP (0.5 μg), (4) MNC (0.5 μg) and (6) EMP (0.5 μg).

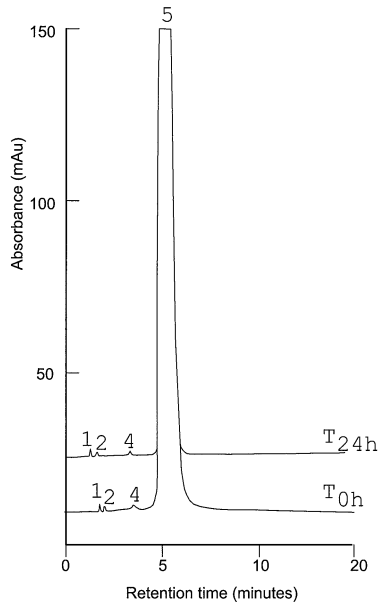

Fig. 5 Typical chromatogram of bulk drug of SLD (20 μg) stored in the mobile phase at ambient conditions for 0 h (T_{0h}) and 24 h (T_{24h}). For peak identification see Fig. 3.

Table 4 HPLC determination of potential process impurities in the bulk drug of sildenafil

	Impurity	Concentration (% w/w)	RSD, %[a]
1	CMP	0.42	1.67
2	MNP	0.21	1.09
3	AMP	NIL	—
4	MNC	0.36	1.43
5	EMP	NIL	—

a. $n = 6$.

Fig. 5. The amounts of various impurities were determined, and the purity of SLD was calculated. The results are recorded in Table 4. To determine the stability of SLD in the mobile phase, the drug was stored in the mobile phase for 24 h and chromatographed on the following day. The chromatogram is shown in Fig. 5. It can be seen from the chromatograms of Fig. 5, T_{0h} and T_{24h} that no significant change was observed. From these results, it is clear that the method is precise and accurate for the separation and determination of small quantities of some of the process impurities that are generally present in SLD.

Conclusion

A robust and sensitive HPLC procedure has been developed for the rapid determination of SLD and its synthetic impurities *viz*., MNP, MNC, AMP, CMP and EMP. The developed HPLC method is suitable not only for the separation and determination of process impurities, but also for monitoring the synthetic process of SLD. The method is thus suitable for the process development and quality assurance of SLD and related products.

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