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Solid phase extraction and liquid chromatographic determination of sildenafil and *N*-demethylsidenafil in rat serum with basic mobile phase

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Abstract

HPLC method for the determination of sildenafil and its metabolite (*N*-demethylsidenafil) in rat serum has been developed. The technique included a solid phase extraction of the serum samples on a [poly(divinylbenzene-co-*N*-vinylpyrrolidone)] solid phase extraction sorbent. After conditioning, the cartridge was loaded with 0.5 mL of buffered serum containing internal standard. Elution was made with 1 mL of acetonitrile. After evaporation of the eluates to dryness and reconstitution with methanol, the samples were analyzed on Kromasil C_{18} column phase with phosphate buffer 0.05 M/acetonitrile: 54/46, pH 8. Detection was carried out using a photodiode array detector. For sildenafil and demethylsildenafil, full validation of the proposed method was provided (linearity range, calibration curves, average extraction efficiency; average intra-day and interday variabilities, limit of detection, limit of quantification, specificity). The proposed method was successfully utilised to quantify sildenafil and *N*-demethylsidenafil in rat serum for a pharmacokinetic study.

Keywords: Column liquid chromatography; Basic mobile phase; Solid phase extraction; Photodiode array detector; Sildenafil; Demethylsildenafil

1. Introduction

Sildenafil (1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine) is used as an oral agent to treat male erectile dysfunction [1–3]. It is a selective inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDE5). Sildenafil citrate is metabolized and demethylated to give UK-103, 320 having a similar potency to sildenafil and hence may contribute to pharmacological effects. Therefore, it is essential to evaluate the two compounds in serum.

Many techniques were used to determine sildenafil citrate in pharmaceuticals, such as spectrophotometric methods [4] or HPLC. The determination of sildenafil citrate in pharmaceuticals was made with HPLC using C_{18} [5] or monolithic columns [6]. Daraghmeh et al. [7] reported an HPLC technique for the determination of sildenafil citrate and its related substances. Recently, Abd-Elbary et al. [8] proposed an HPLC procedure for the determination of sildenafil citrate in bulk and in formulations. Structure elucidation of sildenafil analogues in herbal products was made by Blok-Tip et al. [9].

There are some papers in the literature reporting the determination of sildenafil in pharmaceuticals [10] or in serum samples using micellar electrokinetic [11,12], capillary zone electrophoresis [13], gas chromatography [14,15] or HPLC [16,17]. A high-performance liquid chromatographic (HPLC) analysis of sildenafil and one of its metabolite UK-103, 320 (*N*-demethylsildenafil) in serum was reported using automated sequential trace enrichment of dialysates (ASTED) system to prepare serum samples [18]. Another method using narrow-bore column switching has also been used to this for the simultaneous determination of sildenafil and its active metabolite in serum [19]. Liquid chromatographic method of sildenafil with a detection limit of 10 ng mL^{-1} was proposed by Sheu et al. [20]. Liquid chromatography–tandem mass spectrometry was used for simultaneous assay of sildenafil and desmethylsildenafil in human serum [21–23].

It is important to note that both sildenafil and its demethylated metabolite UK-103, 320 (Fig. 1) have basic functional groups with a pK_a, value of 8.7 [18]. It is well known that difficulties may arise during the analysis of compounds with basic properties due to the adsorption exposed residual silanols of silica HPLC

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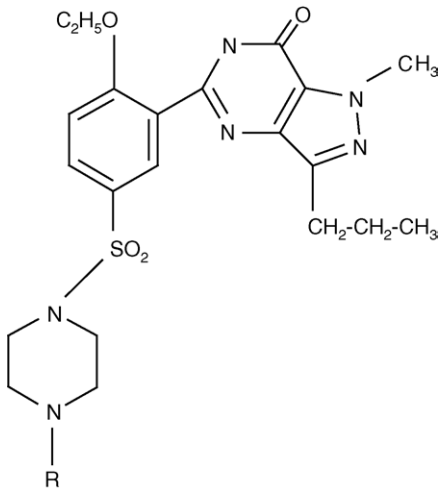

Fig. 1. Chemical structure of sildenafil (R = CH_3) and *N*-demethylsidenafil (R = H).

column. These interactions induce peak tailing, which can affect resolution, sensitivity, and reproducibility. A new approach consists in developing new stationary phases, especially designed to avoid secondary interactions between basic compounds and free silanols. Some of these supports even if silica based, are chemically stable over a wide pH range.

In the other hand, sildenafil and its metabolite are highly and weakly protein binding [24]. Solid phase extraction can give an alternative solution to isolate the basic SC and its metabolite.

Throught the literature [11,14,21,25–28], before solid phase extraction, it appeared that it was not necessary to carry out a special treatment of serum samples to unbound sildenafil and its metabolite. To isolate sildenafil and its metabolite from serum, liquid–liquid extraction [20] or solid–liquid extraction were used. Solid phase extractions were performed on cartridges C_8 [21] or C_{18} bonded silica [11,14,25–28]. The proposed SPE procedures were difficult to use and time consuming. This classical C_{18}-bonded silica phase cannot endure to strong apolar organic solvents, contrary to the polymeric sorbents. Modern porous polymer sorbents stable in a large pH range (pH 1–11) are generally copolymers of styrene and divinylbenzene processed to enhance their properties for SPE [29–32]. The large surface areas (700–1200 m^2/g) and the porous structure of the highly crosslinked polymers result in higher retention with a compatibility with aqueous and organic solvents. Extend of their properties can also be increased by light surface modification with polar functional groups [31,33]. The same goals are achieved by a macroporous poly(divinylbenzene-co-*N*-vinylpyrrolidone) polymer (Oasis HLB), which has a surface area of about 800 m^2/g [34]. Several references describing the successful biological applications of this sorbent such in the extraction of methadone and benzodiazepine [35], linezolid [36], epirubicin [37], metroprolol [38], lanzoprazole and its metabolites [39], celecoxib [40]. In this work, a SPE cartridges based on poly(divinylbenzene-co-*N*-vinylpyrrolidone) were used to direct extracting SC and its metabolite. HPLC technique with a basic mobile phase was developed.

2. Experimental

2.1. Reagents

Acetonitrile and methanol of chromatographic grade were from Fluka (Switzerland). Ultra pure water was made by the Milli-Q ultra pure system (Millipore, USA). Sildenafil and *N*-demethylsidenafil were kindly gift by Pfizer (USA), phenacetin (internal standard) was from Sigma (USA).

2.2. Chromatographic instrumentation

Waters chromatograph with a 600E pump, 7625i Rheodyne injector with 20 μL sample loop, Waters diode array detector 991 was used. Separations were carried out on a Kromasil C_{18} (250 mm × 4.6 mm) proceeded with a Kromasil C_{18} guard column 1 × 0.4 cm both from Interchim (France). Several mobile phases were tested in isocratic mode. They were made from phosphate buffer 0.05 M and acetonitrile. According to McCalley and Brereton [41], pH measurement of the aqueous buffer was made after to organic modifier addition because the modifier can have a considerable effect on buffer, solute and silanol ionisation.

Flow rate was fixed to 1 mL min^{-1}. Data were collected with Millenium 32 program (Waters). Quantitation was made at 300 nm.

2.3. Collection of the samples

Serum samples were collected from rats of Wistar race (mean weight, 200 g) which received orally 1 mg kg^{-1} of sildenafil suspension. After 0.5, 2, 3, 4, 6, 8, 11 and 24 h, blood samples were collected from the orbital venous plexus. Serum samples were separated by centrifugation at 6,000 rpm for 15 min. They were stored at −20 °C and allowed to defrost at 25 °C prior to use.

2.4. Sample extraction procedure

The solid phase extractions of the samples were carried on poly(divinylbenzene-co-*N*-vinylpyrrolidone) cartridge (Oasis HLB, 60 mg) from Waters. First, conditioning was made by flushing the cartridge with 1 mL of methanol and 1 mL of water. A mixture of 0.5 mL of serum containing phenacetin 20 μg mL^{-1} and 0.1 mL of K_2HPO_4 0.05 M was applied by allowing it to pass trough the bed with a minimal suction, the cartridge was washed with 1 mL of water. Elution was made with 1 mL of acetonitrile. The sample was then evaporated to dryness under nitrogen at 30 °C and reconstituted with 500 μL of methanol. An amount of 20 μL were injected into the chromatograph.

2.5. Extraction recovery

The extraction recoveries were determined by comparing the peak areas of the extracts of spiked serum samples with those

obtained by direct injection of the same amount of sildenafil and *N*-demethylsidenafil.

2.6. Calibration graph

Stock solutions of sildenafil and *N*-demethylsidenafil (200 μg mL^{-1} each) and internal standard (1 mg mL^{-1}) were prepared separately in methanol. Calibration samples of sildenafil and *N*-demethylsidenafil (0.01–2 μg mL^{-1}) and internal standard (20 μg mL^{-1}) were prepared by adding varying volumes of stock solution of sildenafil and *N*-demethylsidenafil and constant volume of internal standard in appropriate volume of pooled drug free serum. The peak area ratio of sildenafil and *N*-demethylsidenafil to internal standard was measured, and a calibration curve was obtained from the least-squares linear regression of the peak area ratio with spiked concentrations. The regression lines were used to calculate concentrations of sildenafil and *N*-demethylsidenafil in the unknown serum samples. Three injections per concentration were carried out for the establishment of the calibration graphs.

2.7. Limit of detection (LOD), limit of quantification (LOQ)

The detection limit (LOD) of sildenafil and *N*-demethylsidenafil was estimated as the drug amounts in serum which corresponded to three times the base line noise. The limit of quantification (LOQ) was determined as the lowest concentration of the calibration curve.

2.8. Precision and accuracy

For spiked serum with different amounts of sildenafil and *N*-demethylsidenafil and constant concentration of internal standard, the inter-run precision of the selected method was estimated by calculating relative standard deviation (R.S.D.) of the concentrations measured at different days ($n = 6$), the intra-run precision was determined with the same way on the same day ($n = 10$). The accuracy was calculated as % bias.

2.9. Selectivity, specificity

To evaluate possible endogenous interferences, five rat serum blanks were analyzed with the proposed procedure.

3. Results and discussion

3.1. Mobile phase selection

Various mobile phases with different compositions and pH were tried to elute sildenafil, *N*-demethylsidenafil and internal standard.

3.2. Influence of the mobile phase pH

Using a constant mobile phase composition (phosphate buffer:acetonitrile, 45:55 (v:v)) adjusted to different pH, sildenafil, its metabolite and internal standard were chromatographied. Results related to the influence of pH are shown in Fig. 2. For the three compounds, It appeared clearly that mobile phase with pH 8 gave the smallest tailing factors, the highest plate numbers and a convenient capacity factors. It is important to note the remarkable tailing factors obtained at pH 8, and interactions of sildenafil or its metabolite with eventual free silanols were minimized under these conditions. This pH was selected for the next pilot investigations.

3.3. Influence of the mobile phase composition

Mobile phases with various amounts of acetonitrile and phosphate buffer but with a constant pH 8 were tested. Mobile phase

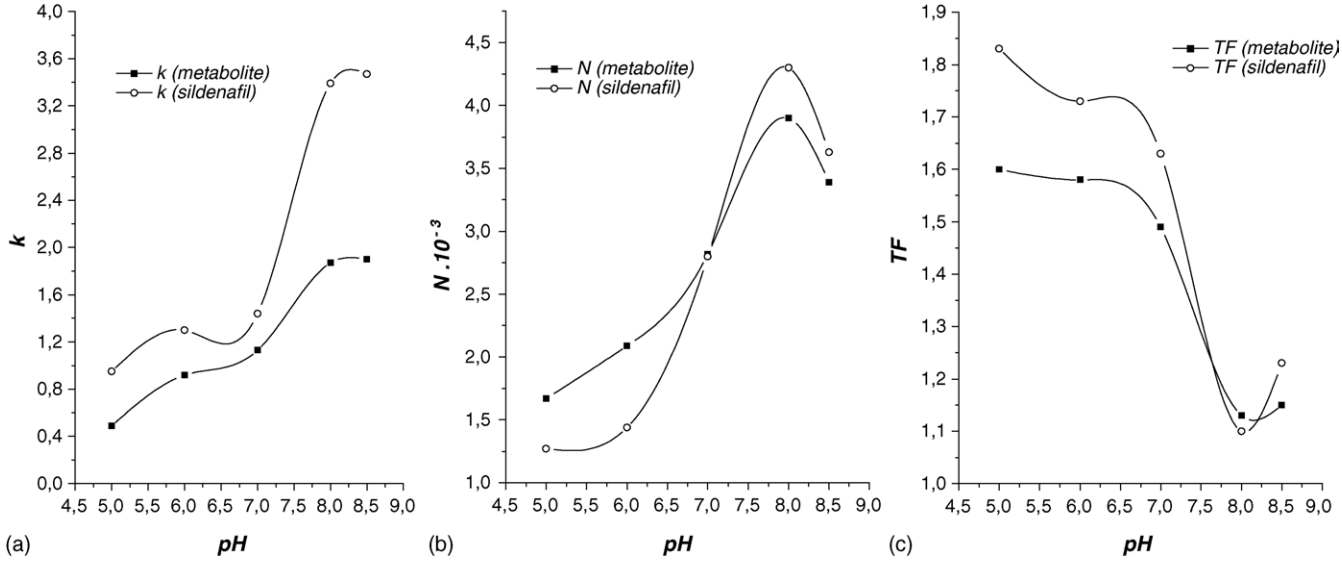

Fig. 2. Effect of the mobile phase pH on the chromatographic parameters (constant composition, phosphate buffer:acetonitrile, 45:55 (v:v)); *k*, capacity factor (a); *N*, plate number (b); TF, tailing factor (c).

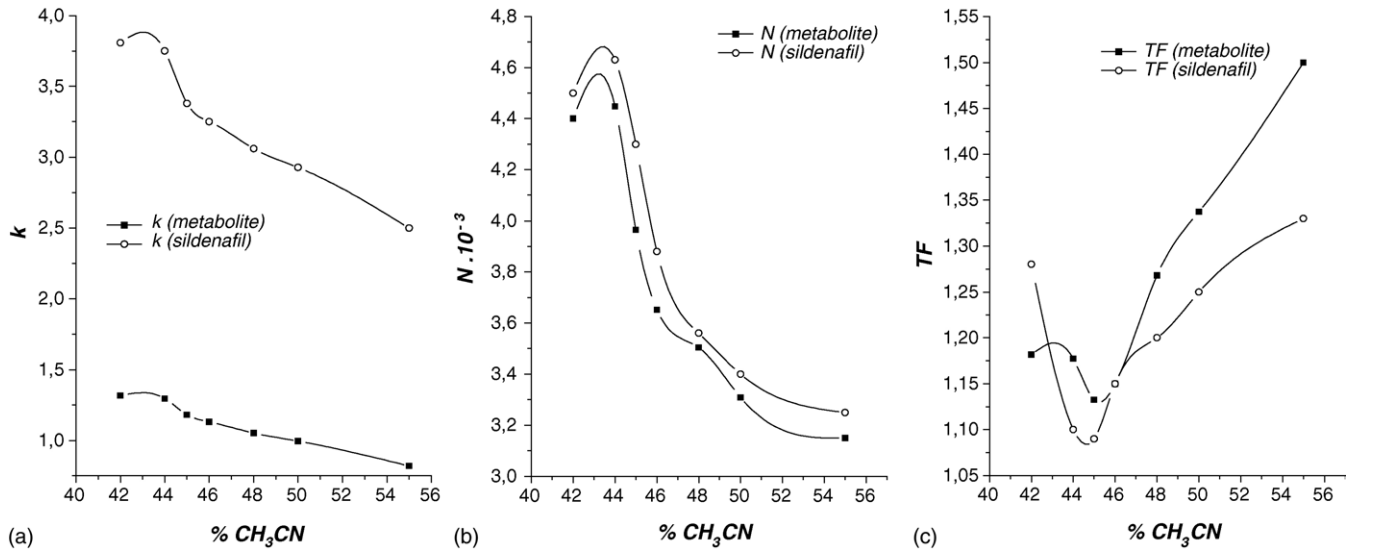

Fig. 3. Effect of the mobile phase composition on the chromatographic parameters at constant pH 8; *k*, capacity factor (a); *N*, plate number (b); TF, tailing factor (c).

selection was based on peak parameters (capacity factor, *k*; efficiency, *N*; tailing factor, TF), easy of preparation and cost.

Fig. 2 showed pH influence on *k* (Fig. 2a), *N* (Fig. 2b), TF (Fig. 2c). For sildenafil and its metabolite, the highest values of plate number was obtained at pH 8 (Fig. 2b) with the lowest tailing factor (Fig. 2c). The difference between their corresponding *k* were also the highest inducing a remarkable selectivity of the chromatographic conditions at pH 8.

In a second step, we investigated the role of acetonitrile composition on the same parameters at pH 8. For the two compounds, results displayed in Fig. 3 showed a maximum of *N* at 44% of acetonitrile (Fig. 3b), a minimum of TF at 45% of CH_3CN. After careful comparison of many columns and HPLC conditions, an acceptable HPLC condition was established with a mobile phase contained: phosphate buffer 0.05 M:acetonitrile, 54:46 (v:v), adjusted to pH 8. Under these conditions, there was no real loss of *N* and TF, sildenafil and its metabolite was eluted forming symmetrical peak and well separated from the solvent front with a convenient sildenafil retention time.

3.4. Assay validation of the method

3.4.1. Calibration graph

Calibration curves were obtained by least-squares linear regression analysis of the peak area ratio *y* of analyte/internal standard versus sildenafil and UK-103, 320 concentration *x* ($\mu g\,mL^{-1}$) were linear with a correlation coefficients of 0.9981 for sildenafil and 0.9932 for the metabolite over the range of 0.010–2 $\mu g\,mL^{-1}$. The corresponding equations were $y = (0.093 \pm 0.005) + (0.159 \pm 0.003)x$ for sildenafil and $y = (0.103 \pm 0.009) + (0.133 \pm 0.003)x$ for its metabolite.

3.4.2. Recovery, precision and accuracy

The recoveries of sildenafil and *N*-demethylsidenafil from spiked serum samples were calculated by comparing peak areas at low, medium and high concentration levels with those obtained from the analysis of corresponding standard dilutions in mobile phase injected directly. The corresponding values were shown in Table 1 which indicated a satisfying recovery of sildenafil and *N*-demethylsidenafil in high, medium or low concentrations. Recoveries obtained with Oasis cartridge gave an average value exceeding 95%. The procedures using C_{18} cartridges [11,14] reach to 90–92% recovery and the authors did not give details about the recoveries for the low concentrations of sildenafil and its metabolite. Jung et al. [42] used C_{18} Sep-Pak cartridge after deproteinization and the extraction recoveries were over 85% in both sildenafil and UK-103, 320. With the C_8 cartridge, recovery of sildenafil and its metabolite decreased to 70% [21].

Table 1
Precision, accuracy and recovery of sildenafil and *N*-demethylsidenafil in spiked serum (SC*, spiked concentration)

SC* ($\mu g\,mL^{-1}$) ($n = 3$)	Sildenafil					*N*-demethylsidenafil				
	Recovery (%)	Bias (%)		R.S.D. (%)		Recovery (%)	Bias (%)		R.S.D. (%)	
		Within-run	Between-run	Within-run	Between-run		Between-run	Within-run	Between-run	Within-run
0.025	93.0 ± 4.3	96.3	95.3	5.3	6.1	94.3 ± 4.7	97.1	96.2	4.9	5.1
0.500	95.8 ± 3.9	97.8	96.4	3.2	3.5	96.3 ± 4.1	97.2	97.0	4.1	3.8
1.00	97.2 ± 2.9	98.1	98.1	2.7	2.9	97.9 ± 3.1	98.4	97.1	3.0	3.2
Average	95.3 ± 3.7	97.4	96.6	3.7	4.2	96.1 ± 4.0	97.6	96.8	4.0	4.0

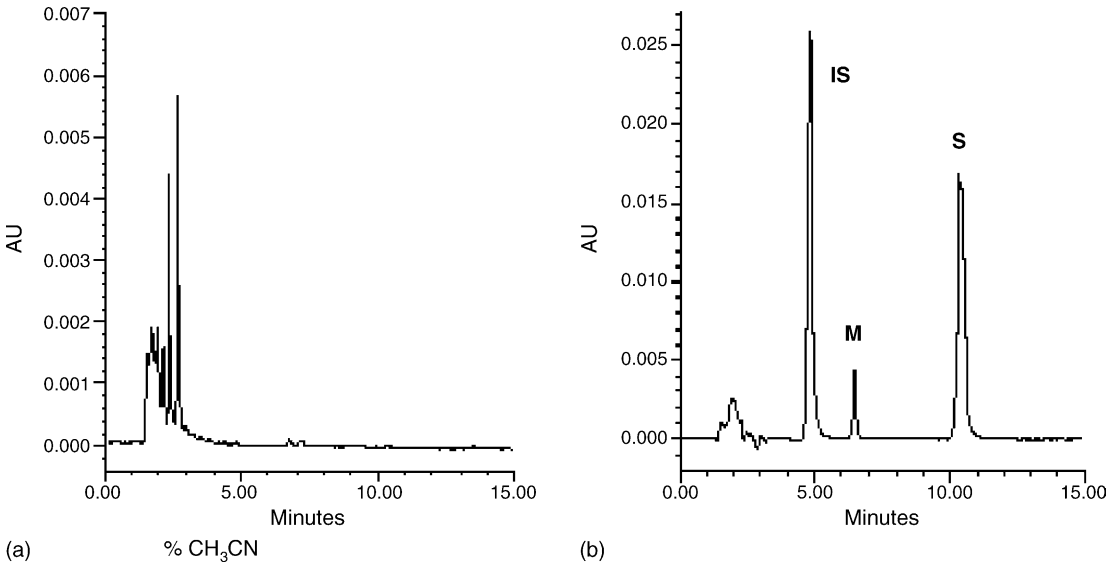

Fig. 4. Representative chromatograms of the extracted blank serum (a) and a rat serum sample (b). IS, internal standard; M, *N*-demethylsidenafil; S, sildenafil.

Table 1 summarized the within and between run precision and accuracy for the determination of sildenafil and *N*-demethylsidenafil in spiked serum as described in Section 2. Within and between run R.S.D.% (precision) by all the methods at low concentration were less than 6.5% and 3% at higher concentrations. The accuracy of the method expressed as % bias for within and between runs was less than 95% at low and high concentrations.

3.4.3. Selectivity, specificity

Fig. 4 showed representative chromatograms of the extracted blank serum (Fig. 4a) and a rat serum (Fig. 4b) sample obtained after administration of sildenafil. No endogenous or extraneous peaks were observed interfering with the assay. Peak purity was further confirmed by photodiode array detection over UV wavelengths from 200 to 400 nm.

3.4.4. Limit of detection (LOD), limit of quantitation (LOQ)

The detection limit (LOD) at signal to noise = 3 and limit of quantification (LOQ) were given in Table 1. For the lower limit of quantitation (LOQ), the lowest concentration of the calibration curve, 0.01 $\mu g\,mL^{-1}$ was taken. The precision and accuracy at this level were within acceptable limits (R.S.D. < 7% sildenafil and *N*-demethylsidenafil).

3.4.5. Application of the method

The proposed method was applied to follow pharmacokinetic of sildenafil in rat. The method was applied to nine subjects showed the concentration profile of sildenafil and *N*-demethylsidenafil with the time in Fig. 5. A maximum serum concentration of sildenafil at $0.59 \pm 0.05\,\mu g\,mL^{-1}$ was reached at 1.05 ± 0.28 h. For the metabolite, C_{max} was $0.24 \pm 0.01\,\mu g\,mL^{-1}$ with 1.60 ± 0.13 h as t_{max}. Terminal elimination half-life was about 4.13 ± 0.51 h for sildenafil and 5.10 ± 0.23 h for its metabolite. Average AUC was

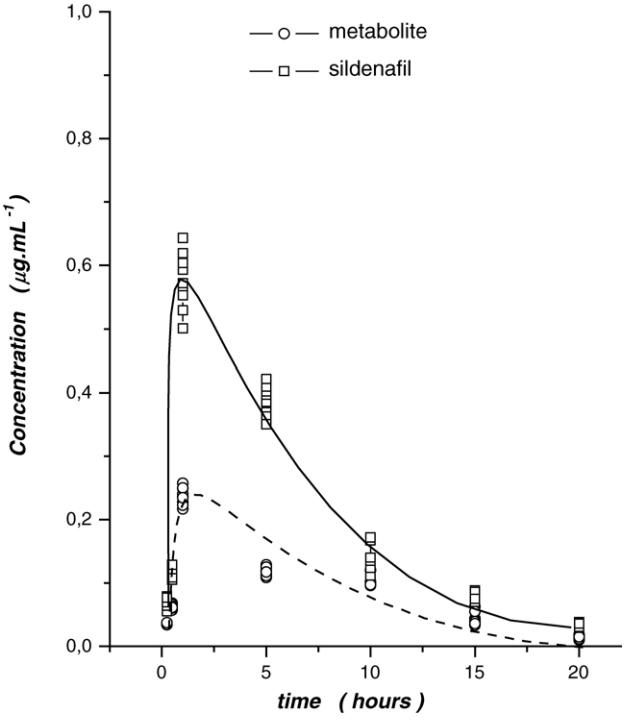

Fig. 5. Serum concentration profile of sildenafil and *N*-demethylsidenafil in rat serum.

$3.99\,\mu g\,mL^{-1}$ h for sildenafil and $1.89\,\mu g\,mL^{-1}$ h for UK-103, 320.

4. Conclusion

In this work, a solid phase extraction (SPE) associated to reversed phase liquid chromatography was described for the determination of sildenafil and *N*-demethylsidenafil in rat serum. The SPE method included the use of [poly(divinylbenzene-co-*N*-vinylpyrrolidone)] cartridge and an easy procedure of serum

treatment. The RP-HPLC procedure used Kromasil C_{18} column phase with phosphate buffer 0.05 M/acetonitrile: 54/46, pH 8. At this pH, remarkable chromatographic parameters, especially tailing factors, were obtained. Detection was carried out with a diode array UV detector. Recovery, precision, sensitivity and linearity were satisfactory in the range studied. The developed method allowed the determination sildenafil and *N*-demethylsidenafil of in rat serum and to followed its pharmacokinetic.

References

[1] A. Chuang, J. Strauss, R. Murphy, W. Steers, J. Urol. 160 (1998) 257–261.
[2] I. Turko, S. Ballard, S. Francis, J. Corbin, Mol. Pharmacol. 56 (1999) 124–130.
[3] D. Umrani, R. Goyal, Indian J. Physiol. Pharmacol. 43 (1999) 160–164.
[4] N.D. Dinesh, P. Nagaraja, N.M. Made Gowda, K.S. Rangappa, Talanta 57 (2002) 757–764.
[5] A. Segall, M. Vitale, V. Perez, M. Palacios, M. Pizzorno, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 1377–1386.
[6] H.Y. Aboul-Enein, M.M. Hefnawy, J. Liq. Chromatogr. Relat. Technol. 26 (2003) 2897–2908.
[7] N. Daraghmeh, M. Al-Omari, A. Badwan, A. Jaber, J. Pharm. Biomed. Anal. 25 (2001) 483–492.
[8] A. Abd-Elbary, N.H. Foda, O.N. El-Gazayerly, Chromatographia 59 (2004) 561–566.
[9] L. Blok-Tip, B. Zomer, F. Bakker, K.D. Hartog, M. Hamzink, J. Ten Hove, M. Vredenbregt, D. De Kaste, Food Addit. Contamin. 21 (2004) 737–748.
[10] J.R. Flores, J.J. Berzas Nevado, G.C. Peñalvo, N. Mora Diez, J. Chromatogr. B 811 (2004) 231–236.
[11] J.J. Berzas Nevado, J. Rodrıguez Flores, G. Castaneda Penalvo, N. Rodrıguez Farinas, J. Chromatogr. A 953 (2002) 279–286.
[12] J.J. Berzas Nevado, J.R. Flores, G.C. Peñalvo, N.R. Fariñas, Electrophoresis 22 (2001) 2004–2009.
[13] W.D. Qin, S.F.Y. Li, Electrophoresis 23 (2002) 4110–4116.
[14] J.J. Berzas Nevado, M.J.V. Llerena, A.M. Contento Salcedo, J.R. Flores, J. Sep. Sci. 25 (2002) 767–772.
[15] A. Pelander, I. Ojanpera, J. Sistonen, I. Rasanen, E. Vuori, J. Anal. Toxicol. 27 (2003) 226–232.
[16] M. Lee, D.I. Min, Therap. Drug Monitor. 23 (2001) 21–26.
[17] H.J. Shim, E.J. Lee, Y.H. Jung, S.H. Kim, S.H. Kim, M. Yoo, J.W. Kwon, W.B. Kim, M.G. Lee, J. Pharm. Biomed. Anal. 30 (2002) 527–533.
[18] J.D.H. Cooper, D.C. Muirhead, J.E. Taylor, R.P. Baker, J. Chromatogr. B 701 (1997) 87–95.
[19] C.K. Jeong, H.Y. Lee, M.S. Jang, W.B. Kim, H.S. Lee, J. Chromatogr. B 752 (2001) 141–147.
[20] M.T. Sheu, A.B. Wu, G.C. Yeh, A. Hsia, H. Ho, J. Chromatogr. B 791 (2003) 255–262.
[21] A. Eerkes, T. Addison, W. Naidong, J. Chromatogr. B 768 (2002) 277–284.
[22] J. Kim, H.Y. Ji, S.J. Kim, H.W. Lee, S.S. Lee, D.S. Kim, M. Yoo, W.B. Kim, H.S. Lee, J. Pharm. Biomed. Anal. 32 (2003) 317–322.
[23] A. Tracqui, B. Ludes, J. Anal. Toxicol. 27 (2003) 88–94.
[24] G.J. Muirhead, M.J. Allen, G.C. James, J. Pearson, D.J. Rance, A.C. Houston, P.M. Dewland, Br. J. Clin. Pharmacol. 42 (1996) 268.
[25] W. Weinmann, M. Bohnert, A. Wiedemann, M. Renz, N. Lehmann, S. Pollak, Int. J. Legal Med. 114 (2001) 252–258.
[26] R. Hyland, E.G.H. Roe, B.C. Jones, D.A. Smith, Br. J. Clin. Pharmacol. 51 (2000) 239–248.
[27] K. Saisho, K.S. Scott, S. Morimoto, Y. Nakahara, Biol. Pharm. Bull. 24 (2001) 1384–1388.
[28] D.K. Walker, M.J. Ackland, G.C. James, G.J. Muirhead, D.J. Rance, P. Wastall, P.A. Wright, Xenobiotica 29 (1999) 297–310.
[29] N. Masque, R.M. Marce, F. Borrull, Trends Anal. Chem. 17 (1998) 384–394.
[30] C.W. Huck, G.K. Bonn, J. Chromatogr. A 885 (2000) 51–72.
[31] M.E. Leon-Gonzalez, L.V. Perez-Arribas, J. Chromatogr. A 902 (2000) 3–16.
[32] N.A. Penner, P.N. Nesterenko, M.M. Ilyin, M.P. Tsyurupa, V.A. Davankov, Chromatographia 50 (1999) 611–616.
[33] J.S. 5Fritz, M. Macka, J. Chromatogr. A 902 (2000) 137–166.
[34] N.C. Dias, C.F. Poole, Chromatographia 56 (2002) 269–276.
[35] H. He, C. Sun, X.R. Wang, C. Pham-Huy, N. Chikhi-Chorfi, H. Galons, M. Thevenin, J.R. Claude, J.M. Warnet, J. Chromatogr. B 814 (2005) 385–391.
[36] J. Toutain, E. Boselli, S. Djabarouti, B. Allaouchiche, F. Xuereb, J.M. Bernadou, B. Ba, M.C. Saux, D. Breilhine, J. Chromatogr. B 813 (2004) 145–150.
[37] R. Li, L. Dong, J. Huang, Analyt. Chim. Acta 546 (2005) 167–173.
[38] J. Fang, H.A. Semple, J. Song, J. Chromatogr. B 809 (2004) 9–14.
[39] M. Miura, H. Tada, T. Suzuki, J. Chromatogr. B 804 (2004) 389–395.
[40] M.H. Guermouche, A. Gharbi, Chromatographia 60 (2004) 341–345.
[41] D.V. McCalley, R.G. Brereton, J. Chromatogr. A 828 (1998) 407–420.
[42] B.H. Jung, D.G. Moon, B.C. Chung, Can. J. Anal. Sci. Spectrosc. 46 (2001) 40–45.