

# Sensitive surfactant-mediated spectrofluorimetric determination of sildenafil†

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Two simple and sensitive surfactant-mediated spectrofluorimetric methods for the determination of sildenafil are proposed in this paper. These methods are based on the interaction of sildenafil with normal micelles of HTAB (hexadecyltrimethylammonium bromide, method A) and the formation of ion-association complexes of sildenafil with SDS (sodium dodecyl sulfate, method B). In both methods, the formed species produce considerable fluorescence enhancement, which allows sildenafil to be quantitatively determined. Linearity was obtained for sildenafil in the concentration range 0.004 to 25  $\mu\text{g mL}^{-1}$  with a detection limit of 0.0012  $\mu\text{g mL}^{-1}$  by method A; and a linearity range of 0.005 to 50.0  $\mu\text{g mL}^{-1}$  with a detection limit of 0.0016  $\mu\text{g mL}^{-1}$  by method B. The proposed methods have been applied to the analysis of bulk drug, tablets, herbal medicine and beverages. Validation processes were performed by recovery studies and statistical analysis with satisfactory results.

## Introduction

Sildenafil, popularly known as Viagra, is a selective inhibitor of cyclic guanosine monophosphate specific phosphodiesterase type 5. Clinically, is an effective drug for treatment of erectile dysfunction (ED) in males. It is also used for pulmonary hypertension, Raynaud's phenomenon, altitude sickness and Duchenne/Becker muscular dystrophy.<sup>1-4</sup> As a selective pulmonary vasodilator, sildenafil improves gas exchange, increasing life expectancy and exercise tolerance; this has encouraged its use in premature infants with severe respiratory failure and children with primary and post-surgical pulmonary hypertension, severe lung fibrosis.<sup>5,6</sup> As can be expected, application of sildenafil in infants requires an exhaustive control and dosage monitoring. On the other hand, sildenafil shows negative interactions with some medicine used for hypertension and ischemic heart disease treatment<sup>7</sup> (e.g., nitroglycerine, doxazosin and terazosin). Unfortunately, ED occurs frequently in patients with these diseases (i.e. up to 60–70%). Since sildenafil was launched on the market as a novel treatment for ED, it has been converted into one of the most widespread drugs of use and abuse. Moreover, sildenafil is found as common adulterant in herbal medicine for treatment of ED and some beverages. The illicit addition of sildenafil in the wide variety of products makes it a potential risk for the society. Therefore, an accurate, sensitive and robust methodology for sildenafil determination is highly required. For

this purpose, several methods have been developed,<sup>8</sup> such as spectrophotometric,<sup>9,10</sup> chromatographic methods,<sup>11-15</sup> MEKC,<sup>16</sup> voltammetric,<sup>17,18</sup> potentiometric<sup>19</sup> and HPLC<sup>20</sup> methods; but determination of sildenafil based on its fluorescence property has not been reported thus far. HPLC with spectrophotometric detection is mostly applied due to its known advantages related to versatility, but often time-consuming sample pre-treatment steps are required.

Surfactants are molecules containing a hydrophobic long-chain with a polar head group which could be ionic or neutral. When these amphiphilic molecules are dissolved in water, they have a tendency to form spontaneous aggregates. Reaching a particular concentration (critical micellar concentration, CMC), surfactant molecules will arrange themselves into organized molecular assemblies, also called micelles. It is widely known the ability of these organized media to provide an appropriate microenvironment able to modify catalytic and luminescence properties of reactants and products. Moreover, surfactants have the advantages of being less harmful to the environment and human health than traditional organic solvents.<sup>21</sup> Before a critical concentration is reached, pre-micellar aggregates may still be formed.<sup>22-24</sup>

In this work, the fluorescence of sildenafil in the presence of hexadecyltrimethylammonium bromide (HTAB) and sodium dodecyl sulfate (SDS) surfactants is studied. Theoretical interaction mechanisms supported by spectral studies are proposed for drug-surfactant systems. Taking into account the great improvement on analytical sensitivity, two new direct methods for quantitative determination of sildenafil in real pharmaceutical samples are developed. Moreover, one of proposed methods has been applied in determining sildenafil in non-registered formulations and common beverages.

## Experimental

### Instrumental

Shimadzu RF-5301PC spectrofluorimeter (Shimadzu Corporation, Analytical Instrument Division, Kyoto, Japan), equipped

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† Electronic supplementary information (ESI) available: Figure 1. Fluorescence emission spectra of sildenafil-HTAB. Figure 2. Influence of HTAB concentration on sildenafil fluorescence intensity. Figure 3. UV-spectra of sildenafil-SDS system. See DOI: 10.1039/b9ay00208a

with a Xenon discharge lamp and 1 cm quartz cells was used for the fluorescent measurements.

Beckman spectrophotometer with 10 mm optical path cells was used to record UV-vis absorption spectra.

A pH meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) Model EA940 with combined glass electrode was used for monitoring pH adjustment.

## Reagents

Sildenafil (as citrate) was kindly provided by Gador S.A. (Buenos Aires, Argentina). Reagents of analytical grade were used: SDS and HTAB were purchased from Tokyo Kasei Industries (Chuo-Ku, Tokyo, Japan). Tris (Mallinckrodt Chemical Works, New York, Los Angeles, St. Louis, USA), NaOH and HCl (Merck, Darmstadt, Germany). High-purity water was obtained from a Millipore (Milford, MA, USA) Milli-Q Plus System.

## Assay solutions

Sildenafil standard solution containing 2.0 mg mL<sup>-1</sup> was prepared dissolving the reagent with ultra pure water. This solution was stable for at least two weeks stored at room temperature.

10.0 mM SDS and 10.0 mM HTAB were prepared with an adequate weight of reagents and dissolving in ultra pure water. A 1 × 10<sup>-2</sup> mol L<sup>-1</sup> HCl solution was prepared by diluting an adequate volume of concentrated acid with ultra pure water.

The pH values in optimization stage were adjusted by the addition of solutions of NaOH(c) or HCl(c) until the target pH value was reached.

## Sample solutions

- **Pharmaceutical formulations.** Five tablets of VORST® (purchased by Bernabó Pharmaceutical Industry, Buenos Aires, Argentina) and MAGNUS® (purchased by Sidus S.A. Buenos Aires, Argentina) containing 25 and 50 mg sildenafil were weighed and finely powdered. Portions of the powder equivalent to 25 and 50 mg of sildenafil respectively, were dissolved in water and solid residues were separated by filtration. The solutions were then transferred to 50 mL flasks and diluted to the final volume with ultra pure water.

- **Herbal medicines.** Aqueous extract of *Lycopodium saururus* was obtained by infusion of 5 g (dry weight) of the commercially available medicine with 80 mL of boiling ultra pure water. After 5 min., the mixture was filtered and made to 100 mL with ultra pure water.

Aqueous extract of mixture herbal (*Haploppapus baylahuen*, *Lycopodium saururus*, *Baccharis articulata*, *Thymus vulgaris* and *Salvia apiana*) was obtained by infusion of 5 g (dry weight) of this commercially available mixture with 80 mL of boiling ultra pure water. After 5 min., the mixture was filtered and made to 100 mL with ultra pure water.

- **Beverages.** 1 g of orange juice powder was weighted and 80 mL of ultra pure water were added. The mixture was then filtered and made to 100 mL.

An infusion of tea was obtained employing a commercial tea bag (containing 2 g of dry weight *Camelia sinensis*) and adding 80 mL of boiling ultra pure water. After 5 min., the mixture was filtered. The filtered liquid was made to 100 mL with ultra pure water.

## General procedure

**Method A.** Aliquots of standard containing between 0.005 and 50.0 µg mL<sup>-1</sup> of sildenafil or sample solution, 1 mL of HTAB (10 mM) and 1 mL of buffer Tris (0.01 M, pH 11) were added to graduated volumetric flasks and then diluted to 10 mL with ultra pure water. The fluorescence signals were recorded at 290 nm and 435 nm as excitation and emission wavelength, respectively.

**Method B.** Aliquots of standard containing between 0.005 and 50.0 µg mL<sup>-1</sup> of sildenafil or sample solution, 0.40 mL of SDS (10 mM) and 0.10 mL HCl (1 mM) were added to graduated volumetric flasks and then diluted to 10 mL with ultra pure water. The fluorescence signals were recorded at 315 nm and 415 nm as excitation and emission wavelength, respectively.

## Results and discussions

Micelles are highly dynamic aggregates and rates of aggregation processes are nearly at the diffusion-controlled limit.<sup>25,26</sup> When surfactant concentration is very low, these monomers behave like independent molecular entities. As surfactant concentration increases, they tend to come closer to each other and form aggregates of different size and shape, this process being thermodynamically favored. Micelles formation can be studied by several methods<sup>27</sup> based on the fact that at the vicinity of the CMC occurs a sharp change in experimental parameters: surface tension, absorbance, rate of reaction, conductivity, osmotic pressure, *etc.* Fluorescence spectroscopy<sup>28</sup> is an adequate instrumental technique for CMC determination. Additionally, micellar microenvironment can produce an enhancement on fluorescence emission and improves the sensitivity of analytical methodology.

Sildenafil has a basic functional group (NH-piperazine) with a pK<sub>a</sub> value of 8.7 and a second pK<sub>a</sub> of 9.6–10.1 due to NH-amide.<sup>29,30</sup> Due to its acid-basic characteristics, sildenafil can adopt a positive or negative charge, depending on the pH of the medium.

## Sildenafil-HTAB system

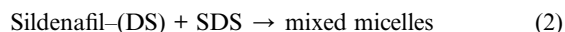
When cationic surfactant HTAB is added above a certain limit, the positively charged micellar aggregates begin to form. In alkaline medium, a micellar interface with positive zeta-potential would be the preferred location for the negatively charged molecule of sildenafil to be bound, through electrostatic forces of attraction.<sup>31</sup> With further addition of HTAB the aggregates grow until they reach the CMC value, where micelles coexist with surfactant monomers.<sup>32</sup> When the monomer concentration is far from the CMC value, the fluorescent intensity does not increase significantly with varying C<sub>HTAB</sub>. But near to the CMC value, fluorescence signal of sildenafil is enhanced, indicating that drug molecules are bound to the micellar aggregate. The corresponding fluorescence enhancement reaches its maximum when

HTAB micelles are formed ( $C_{\text{HTAB}} = \text{CMC}$ ). This spectral behaviour is attributed to the change in microenvironment surrounding sildenafil, which is quite different from bulk water. To determine the CMC value (see supporting information†), the rising part and plateau were fitted with linear functions. These two fitted lines cut each other at a point corresponding to the CMC,<sup>33</sup> which was  $\sim 0.80$  mM, this is in good agreement with the literature reported value from pure aqueous medium.<sup>21</sup>

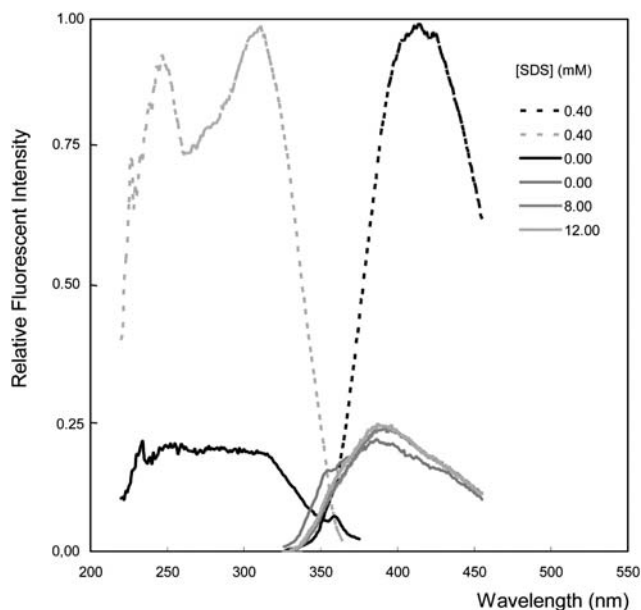
### Sildenafil–SDS system

Since early reports, researchers have already referred to anomalies in the behavior for diluted surfactants; many dye–surfactant systems, especially when the dye has opposite charge to the surfactant, form dye–surfactant aggregates.<sup>34,35</sup>

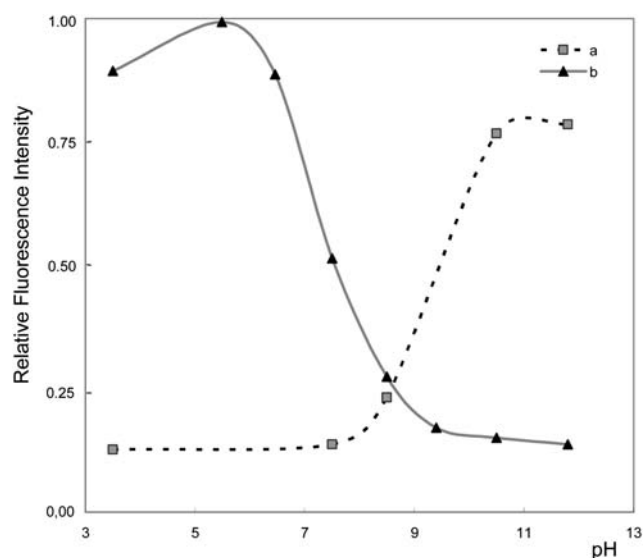
Below to pH 6, sildenafil is found as cationic form. In the presence of diluted anionic surfactant SDS, where SDS monomers prevail (as dissociated form,  $\text{DS}^-$ ), the positively charged molecule of sildenafil is attracted by electrostatic force, forming a sildenafil–DS complex. In Fig. 1, emission spectra of sildenafil–SDS are shown. The emission intensity enhancement accompanied by bathochromic shift, is evidence of sildenafil–DS complex formation. Further addition of SDS produces the formation of some mixed aggregate or micellar structure. This phenomena can be described by these following equations:



When sildenafil–(DS) complex is formed, the free rotation motions of sildenafil molecule are restricted. Thus, rigidity on sildenafil structure facilitates the fluorescent emission processes from the excited state instead of other nonradioactive relaxations.



**Fig. 1** Fluorescence spectra of sildenafil–SDS system.  $C_{\text{sildenafil}} = 40 \mu\text{g mL}^{-1}$ ; pH 5;  $C_{\text{SDS}} = 0.0\text{--}12.0$  mM.  $\lambda_{\text{exc}} = 315$  nm;  $\lambda_{\text{em}} = 415$  nm.



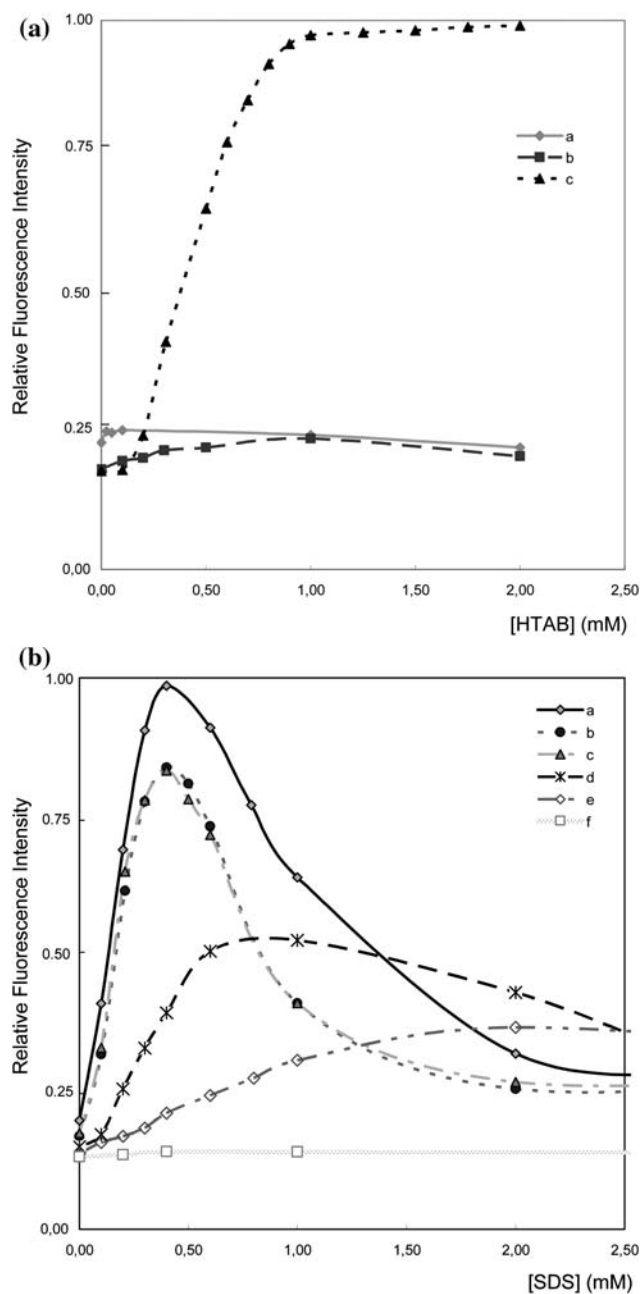
**Fig. 2** Effect of pH on sildenafil–surfactants systems.  $C_{\text{sildenafil}} = 40 \mu\text{g mL}^{-1}$ . a:  $C_{\text{HTAB}} = 1.0$  mM,  $\lambda_{\text{exc}} = 290$  nm;  $\lambda_{\text{em}} = 435$  nm; b:  $C_{\text{SDS}} = 0.40$  mM,  $\lambda_{\text{exc}} = 315$  nm;  $\lambda_{\text{em}} = 415$  nm.

**Influence of pH and surfactant concentration.** Since sildenafil has acid–basic properties, it is expected that the pH has influence on equilibria of complexes or aggregate formation between the drug and surfactants. In Fig. 2, it can be appreciated for the sildenafil–HTAB system that the fluorescent signal increases as pH increases. In contrast, for sildenafil–SDS system, where pH increases from 3.5 to 11.8, the fluorescent signal decreases. The observed inflexion points in pH curves give the experimental  $\text{p}K_{\text{a}}$  values for each sildenafil–surfactant system. These values are in concordance with those obtained by other authors.<sup>29,30</sup>

In Fig. 3, plots of pH influence on sildenafil fluorescence intensity for systems with different surfactant concentrations are presented. Optimal experimental values of pH and surfactants concentration were: for sildenafil–HTAB system, 11.0 at 1.0 mM of HTAB, and for sildenafil–SDS system, 5 at 0.4 mM of SDS.

**Influence of ionic strength.** In order to study the effect of ionic strength on sildenafil–HTAB and sildenafil–SDS systems, different concentrations of inert salts (NaCl, sodium citrate and sodium acetate) were added to the systems. For all tested electrolytes in concentrations below to  $2 \times 10^{-3}$  mol  $\text{L}^{-1}$ , no change in fluorescent signals was observed. Above this concentration, a slight decrease on the fluorescence intensities was observed for both sildenafil–surfactant systems.

**Figures of merit.** Calibration curves of sildenafil were performed under optimal conditions according to those described in general procedure. Data were fitted by standard least-squares treatment and analytical parameters determined for each method are given in Table 1. According to IUPAC definition, the slope of the calibration graph ( $m$ ) represents the sensitivity of calibration. Comparing to spectrophotometric detection for sildenafil assay, proposed methods were approximately 1,000 times more sensitive, and detection limit ( $\text{LOD} = 3\sigma/m$ ) and quantification limit ( $\text{LOQ} = 10\sigma/m$ ) were improved by three magnitude orders. These



**Fig. 3** Influence of pH at different surfactant concentrations. Fig. 3a) a: pH = 3.5; b: pH = 7.5; c: pH = 11 ( $\lambda_{\text{exc}} = 290$  nm;  $\lambda_{\text{em}} = 435$  nm). Fig. 3b) a: pH = 5.5; b: pH = 3.5; c: pH = 6.5; d: pH = 7.5; e: pH = 8.5; f: pH = 11 ( $\lambda_{\text{exc}} = 315$  nm;  $\lambda_{\text{em}} = 415$  nm).  $C_{\text{sildenafil}} = 40 \mu\text{g mL}^{-1}$ .

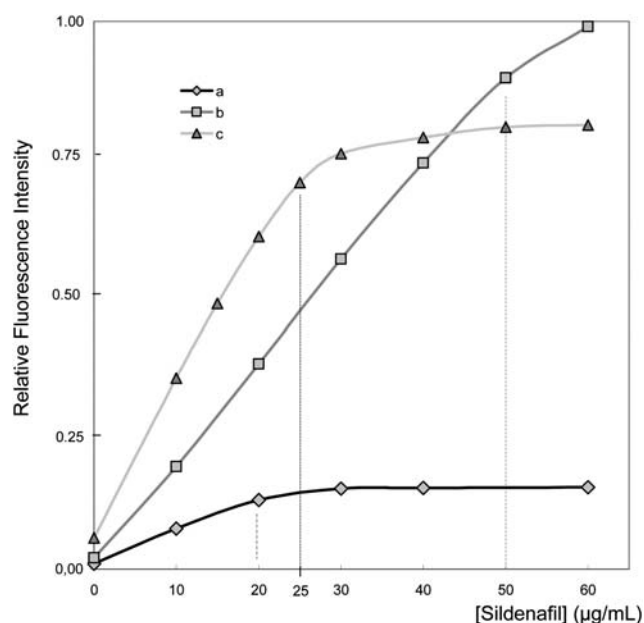
facts represent the potentiality of the proposed methods to compete with the UV-vis spectrophotometry for sildenafil assay, which is the custom method for this purpose.

Fig. 4 shows the calibration curve of sildenafil in the presence and absence of surfactants. As can be seen, the linear range (LOL) for sildenafil in aqueous medium has a linearity limit of  $20.0 \mu\text{g mL}^{-1}$ . In presence of HTAB micelles, this limit was extended to  $25.0 \mu\text{g mL}^{-1}$ ; employing  $0.40$  mM of SDS, this limit was extended up to  $50.0 \mu\text{g mL}^{-1}$ . In both methods, an important improvement in analytical sensitivity was achieved.

**Table 1** Analytical parameters of spectrofluorimetric developed methods and UV-vis spectrophotometric determination of sildenafil

Analytical parameters	Spectrofluorimetry		UV-visible Spectrophotometry
	Method A	Method B	
$\lambda_{\text{max}}$ (nm)	$\lambda_{\text{exc}} = 290$ $\lambda_{\text{em}} = 435$	$\lambda_{\text{exc}} = 315$ $\lambda_{\text{em}} = 415$	225
LOL ( $\mu\text{g mL}^{-1}$ )	$(0.004\text{--}14.8)^a$ $(0.013\text{--}25)^b$	$(0.005\text{--}22.5)^a$ $(0.018\text{--}50.0)^b$	5.6–50.0
Slope ( $\mu\text{g mL}^{-1}$ )	$67.50^a$	$44.37^a$	0.0441
Intercept	$45.47^a$	$24.33^a$	0.03
Correlation coefficient	$0.9980^a$	$0.9998^a$	0.9973
SD of blank ( $n = 6$ )	$0.029^a$	$0.024^a$	0.026
LOQ ( $\mu\text{g mL}^{-1}$ )	$0.004^a$	$0.005^a$	5.60
LOD ( $\mu\text{g mL}^{-1}$ )	$0.0012^a$	$0.0016^a$	1.76

<sup>a</sup> Excitation slit width = 5 nm; emission slit width = 10 nm. <sup>b</sup> Excitation slit width = 5 nm; emission slit width = 5 nm.



**Fig. 4** Calibration curve of sildenafil in absence and presence of surfactants. a: aqueous medium; b:  $0.40$  mM ( $C_{\text{SDS}} \ll \text{CMC}$ ), pH 5,  $\lambda_{\text{exc}} = 315$  nm;  $\lambda_{\text{em}} = 415$  nm; c:  $1.0$  mM ( $C_{\text{HTAB}} \geq \text{CMC}$ ), pH 11,  $\lambda_{\text{exc}} = 290$  nm;  $\lambda_{\text{em}} = 435$  nm.

The deviation of linearity calibration curve of sildenafil in aqueous medium could be explained by the dimerization processes of the drug.<sup>32</sup> This aggregation process may conduce to auto-quenching. The presence of surfactants leads to complex formation, diminishing the probability of dimerization of sildenafil molecules.

Table 2 shows some reported methods for sildenafil determination. The proposed methods have the best detection limits, only exceeded by ESI mass-spectrometry with prior HPLC separation. Additional advantages of the developed methodologies are associated with the instrumental and operational simplicity, adequately sensitivity and having the widest linearity range.

**Table 2** Analytical parameters of reported and proposed methods for sildenafil determination

Method	Detection system	LOL ( $\mu\text{g mL}^{-1}$ )	LOD ( $\mu\text{g mL}^{-1}$ )	Reference
Extractive spectrophotometric methods	UV-visible spectrophotometry	Method A 1.25–25 Method B 1.5–60	0.16 0.18	9
HPLC	UV-visible spectrophotometry	0.01–1	Not available	12
HPLC-MS	Electrospray positive ionization (ESI) mass-spectrometry	0.000125–0.04	0.00005	16
Micellar electrokinetic chromatography	UV-visible spectrophotometry	0.080–0.9	0.017	17
Adsorptive stripping square-wave voltammetry	Voltammetry	0.029–0.32	Not available	18
Polymer membrane sensors	Potentiometry	6.6–600	3.3	19
This work – Surfactant-mediated spectrofluorimetry	Spectrofluorimetry	Method A 0.004–25 Method B 0.005–50	0.0012 0.0016	—

**Table 3** Recovery study of sildenafil in preanalyzed dosage forms

Formulation (mg/tablet)	Label claim (mg)	Added (mg)	Found (mg) $\pm$ RSD (%) <sup>a</sup>		Recovery (%) <sup>a,b</sup>	
			A	B	A	B
Vorst®	25	—	24.98 <sup>c</sup> $\pm$ 1.34	24.66 <sup>c</sup> $\pm$ 0.80	—	—
		5	29.83 $\pm$ 1.50	29.73 $\pm$ 1.16	97.0	101.4
		10	34.95 $\pm$ 1.66	34.67 $\pm$ 1.02	98.7	100.1
	50	—	48.98 <sup>c</sup> $\pm$ 1.52	48.68 <sup>c</sup> $\pm$ 1.16	—	—
		5	54.01 $\pm$ 0.97	53.75 $\pm$ 0.54	100.6	101.4
		10	58.95 $\pm$ 1.36	58.78 $\pm$ 1.12	98.7	101.0
Magnus®	25	—	25.28 <sup>c</sup> $\pm$ 1.80	25.02 <sup>c</sup> $\pm$ 0.90	—	—
		5	30.24 $\pm$ 0.73	30.11 $\pm$ 2.03	99.2	101.8
		10	35.37 $\pm$ 0.66	34.97 $\pm$ 1.64	100.9	99.5
	50	—	50.99 <sup>c</sup> $\pm$ 0.52	50.79 <sup>c</sup> $\pm$ 0.88	—	—
		5	55.95 $\pm$ 0.80	55.87 $\pm$ 1.08	99.2	98.6
		10	60.95 $\pm$ 1.00	60.89 $\pm$ 1.12	99.62	101.0

<sup>a</sup> Average of 6 replicates. <sup>b</sup>  $100 \times [(\text{found} - \text{base})/\text{added}]$ . <sup>c</sup> Base value. A and B = developed methods A and B respectively.

**Validation and application of methods.** In order to study the accuracy, recovery studies were carried out by the standard addition method. Known amounts of analyte (pure drug) at two different concentration levels were added to commercial formulations and determined. Results are shown in Table 3. Obtained results show quantitative recoveries, indicating good accuracy of

**Table 4** Recovery study of sildenafil using method A, in herbal medicines and common beverages

Samples <sup>a</sup>	Added ( $\mu\text{g}$ ) <sup>b</sup>	Found ( $\mu\text{g}$ ) $\pm$ RSD (%) <sup>c</sup>	Recovery (%)
1	5.00	5.04 $\pm$ 2.50	100.86
	10.00	10.06 $\pm$ 1.05	100.60
2	5.00	4.94 $\pm$ 3.00	98.84
	10.00	9.99 $\pm$ 1.09	99.99
3	5.00	5.06 $\pm$ 2.90	101.20
	10.00	10.00 $\pm$ 0.77	100.00
4	5.00	5.07 $\pm$ 2.20	101.40
	10.00	9.96 $\pm$ 0.75	99.67

<sup>a</sup> 1: *Lycopodium saururus*. Sample volume used = 1 mL. 2: Commercial herbal mixture of *Haploppapus baylahuen*, *Lycopodium saururus*, *Baccharis articulata*, *Thymus vulgaris*, *Salvia apiana*. Sample volume used = 0.1 mL. 3: Commercial powder orange juice. Sample volume used = 0.1 mL. 4: *Camelia sinensis*. Sample volume used = 0.1 mL. <sup>b</sup> Final volume = 10 mL. <sup>c</sup> Average of 3 replicates.

the proposed procedures. These results were compared to the UV-vis spectrophotometric method applying student's test, giving  $t_{\text{calculated}}$  of 0.422 and 0.372 for Method A and B, respectively. In view of the calculated  $t$ -test for the analyzed results which was lower than its tabulated value ( $t_{(0.05,5)} = 2.015$ ). It could be presumed that there is insignificant difference between the obtained results applying spectrophotometric and developed methods (95% probability level). Commonly used additives for pharmaceutical dosage form do not interfere with the proposed procedures.

Moreover, the developed methods were applied to sildenafil determination in local herbal medicine for ED treatment and common beverages. Method B presented severe limitations due to spectral matrix interferences; probably, the equilibrium of complex formation between sildenafil and SDS is perturbed by the high salinity levels of samples. Table 4 shows the obtained results of sildenafil quantification by method A. In this case, method A proved to be adequate for sildenafil quantification in non-registered formulations.

## Conclusions

Fluorescent properties of sildenafil have been studied in the presence of HTAB and SDS surfactants. Since sildenafil could adopt cationic or anionic form, the working pH was a fundamental parameter for its interaction with surfactants. Use of surfactants provides a simple means to enhance the fluorescence of sildenafil (5.5-fold and 7.3-fold increases achieved by HTAB and SDS, respectively) as well as avoiding the self-quenching process of the drug. Therefore, improved LOD and extents on linearity of working curve to determine sildenafil were achieved employing these surfactants. Proposed methods use simple reagents and low-cost instruments. They have proper sensitivity to permit sildenafil determination even down to  $0.0012 \mu\text{g mL}^{-1}$ . These methods are simple, sensitive, accurate, and with good precision allowing the analysis of sildenafil in pharmaceutical quality control. Additionally, method A proved to be effective to sildenafil quantification as adulterant in herbal medicine and beverages.

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