# Spectrofluorimetric Determination of Sildenafil: A New Analytical Alternative for Its Analysis

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*Abstract*- **In aqueous solutions, sildenafil presents a very low fluorescence emission and a limited linear range. In presence of a cationic surfactant hexadecyltrim ethylammonium bromide (HTAB, method A) and anionic surfactant sodium dodecyl sulfate (SDS, method B) a great fluorescence enhancement was observed and the linearity range was enlarged. These surfactants-drug interactions lead to the development of two sensitive methods for sildenafil spectrofluorimetric determination. Nature of interactions between sildenafil and surfactants were studied and different parameters which influence these associations were discussed. Sildenafil was quantitatively determined at an emission wavelength of 435 nm and 415 nm by method A and B respectively with detection limit of 0.0012 μg mL$^{-1}$ and 0.0016 μg mL$^{-1}$. The methods have been successfully applied to the analysis of bulk drug, tablets and herbal medicines.**

*Keywords- Pharmaceutical Analysis; Fluorimetric Analysis; Surfactants; Sildenafil*

## I. INTRODUCTION

Among the population exists a common belief that natural ingredients are inherently safer and healthier than synthetic ingredients [1]. Products sold as "dietary supplements" are subject to significantly less regulation and are often not required to have safety testing or government entities approval before they enter the market. Without adequate safeguards and quality-control mechanisms, there is no assurance that products are free of synthetic medications not included on the labels. In fact, many investigators have shown that other purportedly "all-natural" dietary supplements claiming to improve sexual function actually contained synthetic PDE-5 inhibitors like sildenafil or its analogues [2]. Therefore, analytical chemistry plays an important role to the quality control in pharmaceutical industries, not just quantifying the claimed contents but as well impurities and/or adulterants that may be presented in the medicaments [3]. Fluorescence based methodologies for quality control in pharmaceutical and clinical monitoring have the advantages of being highly sensitive and selective, beside the low-cost equipment involved [4-6].
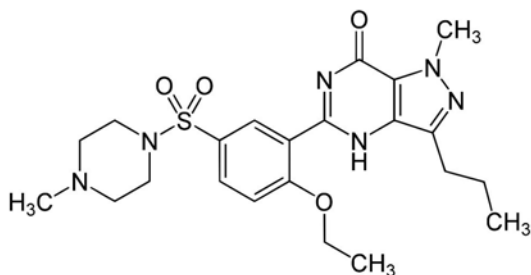


Fig. 1 Molecular structure of sildenafil

Sildenafil (Fig. 1.) is a selective inhibitor of cyclic guanisine monophosphate specific phosphodiesterase type 5. It is the first synthetic effective oral therapy for the treatment of erectile dysfunction with potent vasodilatation effect. Known as Viagra, since its approval by the FDA in March 1998, a large number of prescriptions for this drug have been filled; 148 million pills have been used in Europe and more than one billion worldwide, to fill about 130 million prescriptions [7-8].

Severe cardiovascular event-some leading to death, such as myocardial infarction, ventricular arrhythmia, cardiac arrest, transient ischemic attack, and hypotension—have been reported post-marketing in temporal association with the use of sildenafil [9,7]. The particular social importance of the problem of erectile dysfunction, the effectiveness of the drug and the ease of access to sildenafil (even without medical prescription) has led to cases of uncontrolled use [10-12]. Moreover, the increase in reports of sildenafil (and/or its analogues) found as adulterant in products sold as "herbal medicines" and "dietary supplement" has concerned authorities around the world. This is especially dangerous for patients with preexisting cardiovascular risk and/or taking concomitant medicines for cardiovascular diseases.

The needs of an adequate determination method to detect presence of sildenafil in widespread formulations have lead to the development of a several analytical methodologies [13-19]. Among the analytical techniques, HPLC with spectrophotometric detection is the mostly applied methodology due to its known advantages related to versatility, but often time-consuming sample pre-treatment steps is required. Moreover, the inadequate sensitivity of spectrophotometers as detection system in some cases makes necessary the combination of some analyte-enrichment or derivatization steps.

In this paper, fluorescence behavior of sildenafil in aqueous solution and in presence of HTAB and SDS surfactants is studied. Theoretical interaction mechanisms supported by spectral studies are proposed for the drug-surfactant systems. In light of the great improvement of analytical sensitivity achieved, two new direct methods for quantitative determination of sildenafil in real samples are developed.

## II. INSTRUMENTAL

### A. Instrumentals

Shimadzu RF-5301PC spectrofluorimeter (Shimadzu Corporation, Analytical Instrument Division, Kyoto, Japan), equipped with a Xenon discharge lamp and 1 cm quartz cells were used for the fluorescent measurements.

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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.

### B. Reagents

Sildenafil (as citrate) was kindly provided by Gador S.A. (Buenos Aires, Argentina). Reagents of analytical grade were used: sodium dodecylsulfate (SDS) and hexadecyltrimethylammonium bromide (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.

### C. Assay Solutions

Sildenafil standard solution containing 2.0 mg mL$^{-1}$ 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 5.0 mM HTAB were prepared with an adequate weight of reagents and dissolving in ultra pure water. A $1 \times 10^{-2}$ mol L$^{-1}$ 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.

### D. Sample Solutions

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.

### E. General procedure

#### 1) Method A

Aliquots of standard containing between 0.005 and 50.0 μg mL$^{-1}$ or sample solution of sildenafil, 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.

#### 2) Method B

Aliquots of standard containing between 0.005 and 50.0 μg mL$^{-1}$ or sample solution of sildenafil, 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.

### III. RESULTS AND DISCUSSION

Surfactants are amphiphilic long-chain molecules which depend on the nature of their head group could be ionic or neutral. Dissolved in water, they tend to form spontaneously aggregates and reaching the final size at a limit concentration called critical micelar concentration (CMC). At this instance, the aggregates of surfactants arrange themselves into organized assemblies (called micelles), with particular orientation of their molecules. These organized media has the known ability to modify several physical properties of substances in solution with the additional advantage of being low-toxic and non-pollutant, in comparison with traditional organic solvents [20].

Below the CMC, pre-micelar aggregates may form associations such as surfactant dimmers [21], oligomers [22], dye surfactant entrapments [23], surfactant clusters centered by a metal ion. [24] and substrate-surfactant complexes [25].

The experimental CMC value of surfactants can be calculated by several methods based on the fact that in the vicinity of the CMC there is a sharp change in observables parameters such as surface tension, absorbance, rate of reaction, conductivity, osmotic pressure, etc. Fluorimetry is an adequate instrumental technique for CMC determination [26], because it can be extrapolated from calibrating curve varying surfactant concentration (Fig. 2).
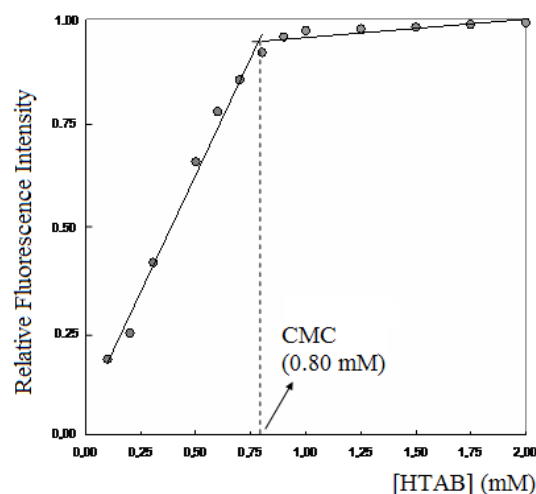


Fig. 2 Influence of HTAB concentration on sildenafil fluorescence intensity

*Csildenafil = 40 μg mL-1; pH 11; CHTAB = 0.0-2.0 mM.*

*λexc = 290 nm; λem = 435 nm*

### A. Optical property of sildenafil

Sildenafil has a basic functional group (NCH$_3$-piperazine) with a p$K$a value of 8.7 and a second pKa of 9.6-10.1 due to NH-amide [27]. Due to its acid-basic characteristics, sildenafil can adopt positive or negative charge, depending on the pH of the medium. It is widely dissolved in aqueous medium and has a strong absorption band at – of UV-vis. These physical parameters have lead to the common detection methods for sildenafil by spectrophotometry in aqueous solution [27]. In this medium, sildenafil presents a poor fluorescence emission. This behavior could be attributed to the rapid deactivation pathway without optical emission, due to the free rotation of its molecular bonds in aqueous medium.

### B. Sildenafil in presence of HTAB

Above CMC of HTAB in solution, cationic micelles of HTAB are formed and coexist with surfactant monomers. The micellar interface with positive zeta-potential would be the preferred location for negatively charged molecule of sildenafil, through the electrostatic force of attraction [28].

The fluorescence spectra of sildenafil in absence and presence of different concentrations of HTAB at pH 11 are shown in Fig. 3. Concentrations of HTAB, far from CMC value, the fluorescence intensity does not increase significantly with varying $C_{HTAB}$. A certain concentration, addition of surfactant monomers increases greatly the fluorescence intensity, reaching the maximum plateau at CMC of HTAB. After CMC, further addition of HTAB did not produce signal enhancement so the fluorescence intensity of sildenafil-HTAB remains constant. This fluorescence enhancement could be attributed to the change in the microenvironment surrounding sildenafil, quit different from the bulk water. In organized media, the motions of sildenafil molecule are restricted, so the emission pathway takes place instead of non-radiation relaxation. In order to determine experimental CMC from Fig. 2, the rising part and the plateau were fitted with linear functions. These two fitted lines cut each other at a point corresponding to the CMC[34], which was at ~0.80 mM, in good agreement with the literature reported value from pure aqueous medium.
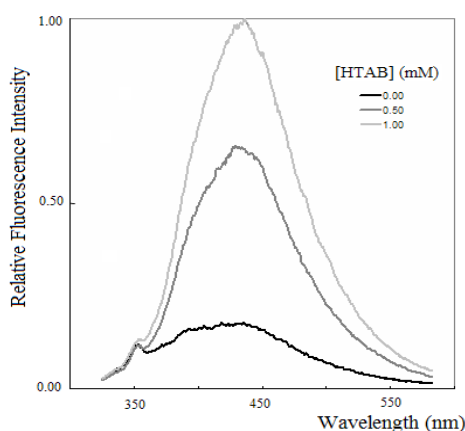


Fig. 3 Fluorescence emission spectra of sildenafil-HTAB

*Csildenafil = 40 μg mL-1; pH 11; CHTAB = 0.0-1.0 mM. λexc = 290 nm; λem = 435 nm*

### C. Sildenafil-SDS system

Anomalies in SDS aggregates formation at pre-micelar concentrations have been already observed by scientists and described in earlier reports. Several dye-surfactant systems – especially when the dye has the opposite charge to the surfactant- form dye-surfactant aggregate [21, 29-30].

Below pH 6, sildenafil is found as cationic form. In presence of diluted anionic surfactant SDS, where the SDS monomers prevail (as dissociated form, SD$^-$), the positively charged molecule of sildenafil is attracted by electrostatic force, forming the sildenafil–DS complex. This complex formed at $C_{SDS}$ << CMC increases greatly fluorescence intensity of sildenafil with an appreciable bathochromic shift (Fig 4).

Further increases of $C_{SDS}$ produce sildenafil-SDS complex dissolution and arrangement of SDS into mixed micelles with decrease of fluorescence intensity and a hipsochromic shift ($\lambda_{max}$ = 392 nm) (Fig 3). After CMC, the fluorescence intensity of sildenafil-SDS maintains constant with further addition of monomers of SDS.

The greater emission intensity and the bathochromic shift ($\lambda_{max\ aq}$ 386 nm; $\lambda_{max\ complex}$ = 415 nm) at pre-micelar region is an evidence of sildenafil-DS complex formation.
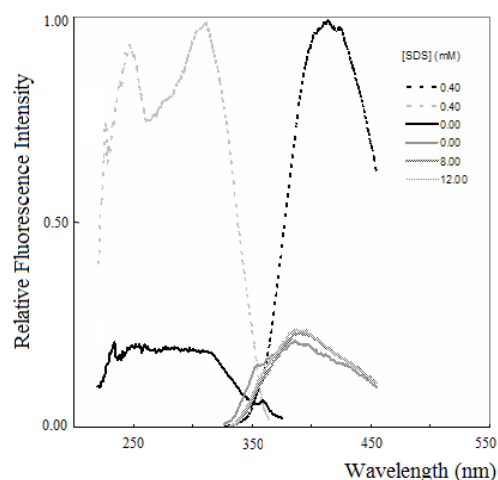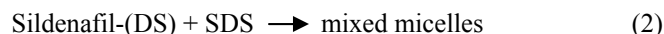


Fig. 4 UV-spectra of sildenafil-SDS system
5a) $C_{SDS}$ = 0 to 0.40 mM; 5b) $C_{SDS}$ = 0.40 to 8.0 mM.
$C_{Sildenafil}$ = 40 μg mL$^{-1}$, pH 5.

The same aggregation behavior has been observed for many cationic dyes and some trivalent metal ions with SDS and this phenomenon can be described by the following equations:

$$Sildenafil^+ + DS^- \longrightarrow Sildenafil\text{-}(DS) \qquad (1)$$

$$Sildenafil\text{-}(DS) + SDS \longrightarrow mixed\ micelles \qquad (2)$$

When the sildenafil-(DS) complex is formed, the free rotation motions of the sildenafil molecule are restricted. Thus, rigidity on sildenafil structure facilitates the fluorescent emission processes from the excited state instead of other non-radian relaxation. The concentration range of SDS to form complex with sildenafil is slight and therefore, the formed complex is quite labile. When some substances compete with sildenafil for monomers of SDS available for complex formation, an important fluorescence decrease will occurred. Therefore, further addition of SDS perturbs the complex formation, because new surfactants aggregates are formed diminishing the surfactants monomers available to form complex with sildenafil.

In order to prove the complex formation, the UV-visible spectrophotometry study for sildenafil-SDS system was carried out. UV-spectra of sildenafil in aqueous medium have showed maximal absorption bands at 230 and 295 nm. As increasing $C_{SDS}$ to 0.40 mM these absorption bands were attenuated gradually with a slight hypsochromic shift for absorption band of 230 nm (Fig. 5a). These spectral changes and the presence of an isosbestic point at 304 nm are evidence of sildenafil–DS complexes formation [31-32]. $C_{SDS}$ above 0.40 mM led to an enhancement on bands intensities (Fig. 5b).
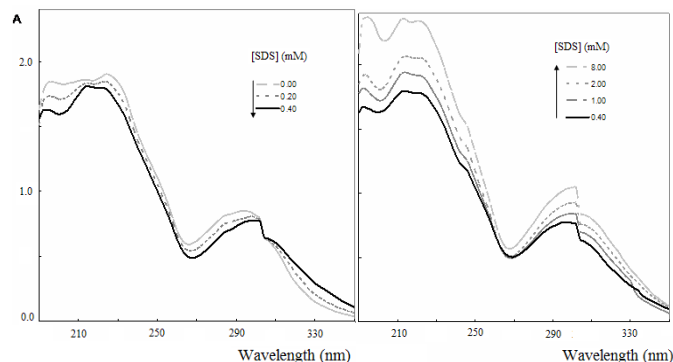
Fig. 5 UV-spectra of sildenafil-SDS system

5a) $C_{SDS}$= 0 to 0.40 mM; 5b) $C_{SDS}$= 0.40 to 8.0 mM. $C_{Sildenafil}$= 40 µg mL$^{-1}$, pH 5.

### D. Influence of pH, surfactant concentration and ionic strength

As described above, sildenafil can adopt either cationic or anionic form, depending on the pH of medium. For aqueous solutions of sildenafil, changes in pH scarcely affect their fluorescence emission. In presence of surfactants the pH of the solution can affects on the interaction between sildenafil and the surfactants, which is mainly electrostatic force of attraction involved.

For sildenafil-HTAB system, fluorescence intensity increases with the increasing of pH, reaching the plateau at pH where sildenafil molecules are expected to be negatively charged (Fig. 6). On the other hand, for sildenafil-SDS system the maximal fluorescence emission is found in the range of pH where sildenafil molecules are mainly positively charged. The experimental pKa values for sildenafil in presence of surfactants were calculated by plots in each pH curves. The observed inflexion points gave results that were in concordance to some reported literature [33].

Influence of pH on fluorescence intensity of sildenafil for both surfactants systems at varying concentrations is shown in Fig. 6. The optimal experimental values of pH and surfactant concentration for sildenafil-HTAB were pH 11.0 at 1.0 mM of HTAB and for sildenafil-SDS were pH 5 at 0.4 mM of SDS.
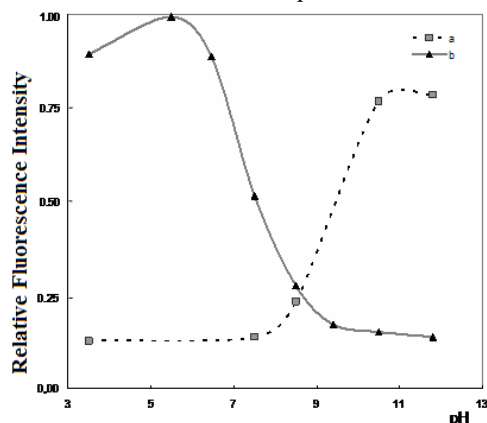


Fig. 6 Effect of pH on sildenafil-surfactants systems

$C_{Sildenafil}$ = 40 µg mL$^{-1}$. a: $C_{HTAB}$ = 1.0 mM, $\lambda_{exc}$ = 290 nm; $\lambda_{em}$ = 435 nm; b: $C_{SDS}$ = 0.40 mM, $\lambda_{exc}$ = 315 nm; $\lambda_{em}$ = 415 nm

Presence of inert salts below to $2 \times 10^{-3}$ mol L$^{-1}$ (NaCl, sodium citrate and sodium acetate in concentrations) has no effect on fluorescence intensity. Above this concentration, a slight decrease on fluorescence intensities was observed for both sildenafil-surfactant systems. Up to $1 \times 10^{-2}$ mol L$^{-1}$ of inert salts, sildenafil-SDS complex was seriously perturbed, and therefore, an important attenuation in fluorescence intensity was observed. Nevertheless, fluorescence emission of sildenafil-HTAB systems was barely affected by increasing salts concentration.

### E. Figures of merit

Calibration curves of sildenafil were performed under optimal conditions according to described in general procedure. Data were fitted by standard least-squares treatment and the analytical parameters determined for each method are given in Table I. According to IUPAC definition, the slope of calibration graph ($m$) represents the sensitivity of calibration. Comparing to spectrophotometric detection for sildenafil assay, the proposed methods were approximately 1.000 times more sensitive, and detection limit (LOD= 3σ/$m$) and quantification limit (LOQ= 10σ/$m$) were improved in three magnitude order. These facts represent the potentiality of proposed methods to compete with the UV-vis spectrophotometry for sildenafil assay, which is the custom method for this purpose.

TABLE I ANALYTICAL PARAMETERS OF SPECTROFLUORIMETRIC DEVELOPED METHODS AND UV-VIS SPECTROPHOTOMETRIC DETERMINATION OF SILDENAFIL

| Analytical Parameters | Spectrofluorimetry | | UV-Visible Phtometry |
|---|---|---|---|
| | Method A | Method B | |
| $\lambda_{max}$ (nm) | $\lambda_{exc}$ = 290 $\lambda_{em}$ = 435 | $\lambda_{exc}$ = 315 $\lambda_{em}$ = 415 | 225 |
| LOL (µg mL$^{-1}$) | (0.004 – 14.8)[a] (0.013 – 25)[b] | (0.005 – 22.5)[a] (0.018 - 50.0)[b] | 5.6 – 50.0 |
| Slope (µg 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 (µg mL$^{-1}$) | 0.004[a] | 0.005[a] | 5.60 |
| LOD (µg mL$^{-1}$) | 0.0012[a] | 0.0016[a] | 1.76 |

[a]: Excitation slit width = 5 nm; emission slit width = 10 nm

[b]: Excitation slit width = 5 nm; emission slit width = 5 nm

As an additional advantage of using surfactant for sildenafil determination is the extension of linearity range (LOL) comparing to aqueous medium. In Fig. 7 the calibration curves of sildenafil in absence and presence of surfactants are presented. Sildenafil in aqueous medium has linearity limit of 20.0 µg mL$^{-1}$ but in presence of HTAB micelles this limit was extended to 25.0 µg mL$^{-1}$ and at 0.40 mM of SDS, this limit was extended up to 50.0 µg mL$^{-1}$. The slope of calibration curves which represent sensitivity of the methodology is several times greater in presence of surfactants than in aqueous medium. The upper limit of calibration curve could be governed by dimmerization process [28] of sildenafil which may lead to fluorescence auto-quenching. The presence of surfactants leads to complex formation, diminishing the dimmerization of sildenafil molecules, and therefore, extends the upper limit of calibration curve.
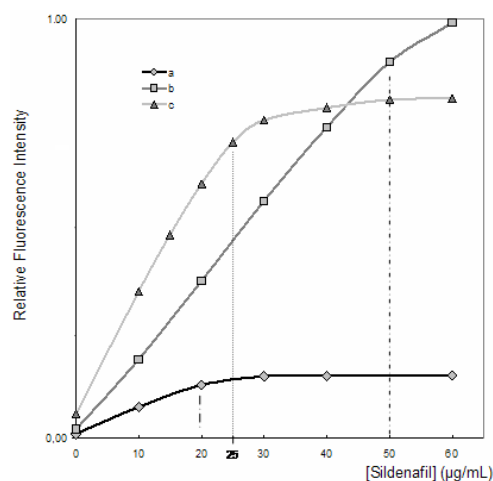


Fig. 7 Calibration curve of sildenafil in absence and presence of surfactants

a: aqueous medium; b: 0.40 mM ($C_{SDS}$ << CMC), pH 5, $\lambda_{exc}$ = 315 nm; $\lambda_{em}$ = 415 nm; c: 1.0 mM ($C_{HTAB}$ ≥ CMC), pH 11, $\lambda_{exc}$ = 290 nm; $\lambda_{em}$ = 435 nm

## F. Validation and application of methods

The accuracy of the present methods was validated by standard addition method and the obtained results were statistically compared to UV-vis spectrophotometric method applying student's t-test. Standard addition method was carry out by spiking known amounts of sildenafil to real pharmaceutical samples (tablets) and determined (Table 3). Average percent recoveries obtained were quantitative, indicating good accuracy of the proposed procedures. In view of the calculated t-test for analyzed results which was lower than its tabulated value, it can presume that there is insignificant difference between the obtained results applying spectrophotometric and the developed methods (95% probability level).

TABLE II RECOVERY STUDY OF SILDENAFIL IN PREANALYZED DOSAGE FORMS

| Sample | Labelled mg/ Tablet) | Added (Mg) | Found (Mg) ± RSD (%)[A] | | Recovery (%)[A, B] | |
|---|---|---|---|---|---|---|
| | | | A | B | A | B |
| Vorst® | 25 | -- | 24.98[c] ± 1.34 | 24.66[c] ± 0.80 | -- | -- |
| | | 5 | 29.83 ± 1.50 | 29.73 ± 1.16 | 97.0 | 101.4 |
| | | 10 | 34.95 ± 1.66 | 34.67 ± 1.02 | 98.7 | 100.1 |
| | 50 | -- | 48.98[c] ± 1.52 | 48.68[c] ± 1.16 | -- | -- |
| | | 5 | 54.01 ± 0.97 | 53.75 ± 0.54 | 100.6 | 101.4 |
| | | 10 | 58.95 ± 1.36 | 58.78 ± 1.12 | 98.7 | 101.0 |
| Magnus ® | 25 | -- | 25.28[c] ± 1.80 | 25.02[c] ± 0.90 | -- | -- |
| | | 5 | 30.24 ± 0.73 | 30.11 ± 2.03 | 99.2 | 101.8 |
| | | 10 | 35.37 ± 0.66 | 34.97 ± 1.64 | 100.9 | 99.5 |
| | 50 | -- | 50.99[c] ± 0.52 | 50.79[c] ± 0.88 | -- | -- |
| | | 5 | 55.95 ± 0.80 | 55.87 ± 1.08 | 99.2 | 98.6 |
| | | 10 | 60.95 ± 1.00 | 60.89 ± 1.12 | 99.62 | 101.0 |

[a] Average of 6 replicates

[b] 100×[(found−base)/added)]

[c] Base value

A and B= developed methods A and B respectively

Additionally, method A was applied to determine sildenafil in herbal medicines infusion and beverages, given satisfactory results (Table 4). For method B we found some limitations for these kinds of samples, maybe due to the disturbance in complex formation equilibrium between sildenafil and SDS in such matrixes. Therefore, method A showed to be more versatile than method B for sildenafil determination in complex matrix samples.

TABLE III RECOVERY STUDY OF SILDENAFIL USING METHOD A, IN HERBAL MEDICINES AND COMMON BEVERAGES

| Samples[a] | Added (μg)[b] | Found (μg) ± RSD (%)[c] | Recovery (%) |
|---|---|---|---|
| 1 | 5.00 | 5.04 ± 2.50 | 100.86 |
| | 10.00 | 10.06 ± 2.50 | 100.60 |
| 2 | 5.00 | 4.94 ± 3.00 | 98.84 |
| | 10.00 | 9.99 ± 1.09 | 99.99 |
| 3 | 5.00 | 5.06 ± 2.90 | 101.20 |
| | 10.00 | 10.00 ± 0.77 | 100.00 |
| 4 | 5.00 | 5.07 ± 2.20 | 101.40 |
| | 10.00 | 10.06 ± 0.75 | 99.67 |

[a] 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.
[b] Final volume = 10 mL. [c] Average of 3 replicates.

Comparing to some reported methods for sildenafil determination, the proposed methodologies showed advantages of their sensitivities (Table IV).

TABLE IV COMPARISON OF SOME METHODOLOGIES FOR DETERMINATION OF SILDENAFIL

| Method | Detection system | LOL ($\mu g\ mL^{-1}$) | LOD ($\mu g\ mL^{-1}$) | Reference |
|---|---|---|---|---|
| Extractive spectrophotometric method | UV-visible spectrophotometry | Method A 1.25–25 Method B 1.5–60 | 0.16 0.18 | [13] |
| HPLC | UV-visible spectrophotometry | 0.01-1 | No available | [14] |
| Micellar electrokinetic chromatography | UV-visible spectrophotometry | 0.080–0.9 | 0.017 | [15] |
| HPLC-MS | Electrospray positive ionization (ESI) mass-spectrometry | 0.000125–0.04 | 0.00005 | [16] |
| Adsorptive stripping square-wave voltammetry | Voltammetry | 0.029 – 0.32 | No available | [17] |
| Polymer membrane sensors | Potenciometry | 6.6 - 600 | 3.3 | [18] |
| Surfactant-mediated spectrofluorimetry | Spectrofluorimetry | Method A 0.004-25 Method B 0.005-50 | 0.0012 0.0016 | This work |

## IV. CONCLUSIONS

Sildenafil in aqueous solutions has a low fluorescence emission, which is greatly enhanced by the presence of surfactants (HTAB and SDS) at certain concentrations.

In presence of HTAB at CMC, fluorescence emission of sildenafil is enhanced about 5.5-fold and 7.3-fold for sub-micellar concentration of SDS and therefore, improves greatly the sensitivity for its determination. Additionally, the self-quenching process of sildenafil was diminished and therefore, linearity of working curve was extended. The parameters

which influence the sildenafil-surfactants interactions were discussed, and the possible mechanisms involved were presented. Based on their interactions, two methods were presented for direct determination of sildenafil in real samples, such as tablets and herbal medicine infusion.

The simplicity, accuracy, economy and versatility of these methods make them suitable for sildenafil analysis for routine quality control tasks using environmental friendly reagents. Moreover, the proposed methodologies could be coupled to HPLC or electrophoresis, in order to improve even more their sensitivity and selectivity.

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