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Research Article

# Analysis of Counterfeit Sildenafil by Validated UV Spectrophotometric Methods

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**ABSTRACT**

First derivative and ratio derivative UV spectrophotometric methods were developed and validated for determination of sildenafil in some adulterated preparations. Sildenafil is commonly adulterated with paracetamol, metronidazole, and glyburide. This work was mainly oriented toward the analysis of some sildenafil preparations that may be adulterated. First derivative spectrophotometry was applied for determination of sildenafil adulterated with paracetamol at λ= 311.8 nm and 265.8 nm for sildenafil and paracetamol, respectively. The linearity ranges were 10-45 µg/mL and 4-12.5 µg/mL for sildenafil and paracetamol, respectively. First derivative spectrophotometry was also applied for the determination of sildenafil adulterated with metronidazole at λ= 319.5 nm and 291.8 nm for sildenafil and metronidazole, respectively. The linearity ranges were 10-42 and 4-16 µg/mL for sildenafil and metronidazole, respectively. For determination of sildenafil adulterated with glyburide, sildenafil was determined by direct spectrophotometry at λ= 328 nm within linearity range 20-100 µg/mL while glyburide was determined by first derivative of ratio spectra at λ= 316.3 nm using 20 µg/mL of sildenafil as divisor with a linearity range of 30-130 µg/mL by adjusting scaling factor at 10 and Δλ= 4 nm. The developed methods were validated as per International Council for Harmonisation (ICH) guidelines.

**Keywords:** Counterfeit, Metronidazole, Paracetamol, Sildenafil, UV spectrophotometry.

## 1. INTRODUCTION

Sildenafil (**Figure 1a**) is a phosphodiesterase-5 inhibitor. It is used for treatment of pulmonary hypertension and erectile dysfunction.[1] Sildenafil is one of the most used drugs. Due to its wide use, it is usually adulterated for different purposes. It is also adulterated by different means as product does not contain active ingredient, product contain wrong amount of active ingredient that lower or higher than the labeled amount or product contain contaminants or other drugs.[1] The active ingredient was found to be (40-100) % or (0 - >200)%.[2] This work mainly oriented for the detection of different adulterants commonly used for adulteration of sildenafil. Paracetamol, metronidazole and glyburide represent the most commonly used drugs in sildenafil adulteration.[2,3] The adulteration of sildenafil has a serious direct and indirect effect. Direct effect due to over dose (more than 100 mg) causes severe hypotension, confusion, and visual disturbance as well as stroke and death.[4] Lower dose leads to low therapeutic effect of the drug. Indirect effect due to other drugs (adulterants) cause drug interaction with sildenafil, hepatic damage in case of paracetamol, increasing toxicity of metronidazole[3] and severe hypoglycemia that may lead to death in case of glyburide.[2] The determination of sildenafil and/or these adulterants is difficult due to the common chemical and overlapped spectral characters of these drugs. In the present work, different UV spectrophotometric techniques could be applied for the analysis of different adulterated sildenafil preparations. No UV

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spectrophotometric method was reported for analysis of sildenafil with paracetamol, metronidazole, or glyburide.

There are several methods for analysis of sildenafil reported in literature such as UV-spectrophotometric methods,[5–7] voltammetry,[8,9] potentiometry,[10,11] and chromatographic method including HPLC,[12–15] LC/MS,[16] GC/MS, [17] LC/MS/MS,[18,19] LC/MS/MS/MS,[19] and LC-ESI-MSI.[20]

Different methods reported for analysis of counterfeit drugs involved spectroscopic methods like IR, NMR, Raman and chromatographic methods as GS/MS spectroscopy and LC/MS but no UV spectrophotometric method was reported for analysis of sildenafil with paracetamol, metronidazole or glyburide.

Paracetamol **(Figure 1b**) is used as analgesic and antipyretic.[21] Metronidazole **(Figure 1c)** is used as antibiotic for anaerobic bacteria and antiprotozoal for definite parasites mainly Entamoeba. Its bactericidal effects contrary to anaerobic bacteria depends on the drug concentration.[22]

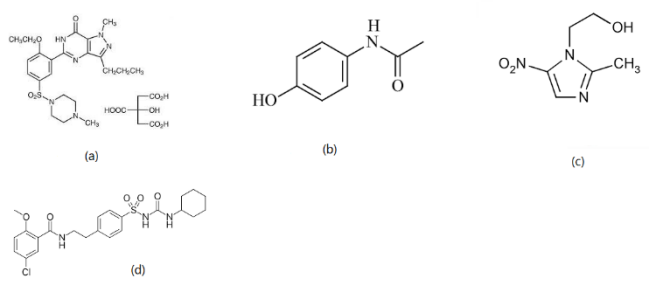


**Figure 1**: Chemical structure of sildenafil citrate (a), paracetamol (b), metronidazole (c), and glyburide (d).

## 2. METHODS

### 2.1. Apparatus and software

UV shimadzu – 1800 240V (Japan) double beam spectrophotometer with UV probe software version 2.33. Quartz cell (1cm) was used.

### 2.2. Materials and reagents

Sildenafil (purity 99.85%), paracetamol (purity99.50%), metronidazole (purity 99.85%) and glyburide (purity 99.70%) were given as gift from Sigma Company for Pharmaceutical Industries, Quesna, Menofia, Egypt. Ethanol and methanol of analytical grade from Sigma Aldrich were used. Avicel (micro crystalline cellulose), croscarmellose, magnesium stearate, $KH_2PO_4$ and hydroxyl propyl methyl cellulose (HPMC) were used as excipients. Distilled water was used.

### 2.3. Standard solutions

#### *2.3.1. For sildenafil and paracetamol*

Standard stock solutions of 250 µg/mL for sildenafil and paracetamol were prepared for analysis of sildenafil adulterated with paracetamol using distilled water as solvent.

Standard working solutions: Transfer 40 mL and 20 mL from sildenafil and paracetamol, respectively into two separate 100 mL volumetric flask and complete volume to the mark with distilled water to obtain concentration 100 µg/mL and 50 µg/mL for sildenafil and paracetamol, respectively.

#### *2.3.2. For sildenafil and metronidazole*

Standard stock solutions of 250 µg/mL for sildenafil and metronidazole were prepared for analysis of sildenafil adulterated with metronidazole using distilled water as solvent.

Standard working solutions: Transfer 40 mL and 20 mL from sildenafil and metronidazole, respectively into two separate 100 mL volumetric flask and complete volume to the mark with distilled water to obtain concentration 100 µg/mL and 50 µg/mL for sildenafil and metronidazole, respectively.

#### *2.3.3. For sildenafil and glyburide*

Standard stock solutions of 200 µg/mL for sildenafil and glyburide were prepared for analysis of sildenafil adulterated with glyburide using ethanol as solvent. The standard solutions were stable for five days in the fridge based on 98% recovery.

### 2.4. Construction of calibration curves

#### *2.4.1. For sildenafil and paracetamol*

Serial dilutions were made by taking different volumes from each standard working solution, then zero order spectra were recorded then first derivative spectra were obtained. The calibration curve was obtained by plotting the amplitude of absorbance versus the corresponding concentrations. The absorbance amplitude for sildenafil was measured at λ= 311.8nm while paracetamol was measured at 265.8 nm. The regression equations were calculated.

#### *2.4.2. For sildenafil and metronidazole*

Serial dilutions were made by taking different volumes from each standard working solution, then zero order spectra were recorded then first derivative spectra were obtained. The calibration curve was constructed by plotting the amplitude of absorbance versus the corresponding concentrations. The absorbance amplitude was measured at λ= 319.5nm for sildenafil and at 291.8 nm for metronidazole. The regression equations were calculated.

### *2.4.3. For sildenafil and glyburide*

Serial dilutions were made by taking different volumes from each standard working solution, then zero order spectra were recorded then first derivative of the ratio spectrum of glyburide was obtained, then plot the calibration curve by absorbance versus concentration for sildenafil at λ= 328nm and amplitude of absorbance versus concentration for glyburide at λ= 316.3nm using 20 µg/mL of sildenafil as divisor. The regression equations were calculated.

## 2.5. Preparation of synthetic mixture

Tablet simulated to that adulterated was prepared by mixing sildenafil 50 mg, adulterant 50 mg (paracetamol or metronidazole or glyburide), and excipients: avicel (micro crystalline cellulose) 229 mg, croscarmellose 7mg, magnesium stearate 3.5mg, KH2PO4 (3.5mg) and HPMC (hydroxyl propyl methyl cellulose) 7mg. The amounts of excipients were added according to Handbook of Pharmaceutical Excipients.[23] Then this mixture was dissolved in methanol, sonicated for 15 minutes, then transferred into 100ml volumetric flask and complete the volume to the mark with methanol, then filter by using Whatman filter paper. From the filtrate transfer 20 mL into 100 mL volumetric flask, complete volume to the mark with solvent (distilled water in case of paracetamol and metronidazole or ethanol in case of glyburide). Suitable dilutions were made and measured.

# 3. RESULTS AND DISCUSSION

## 3.1. Method development

### *3.1.1. For sildenafil and paracetamol*

First derivative method was adjusted; several trials were done for selection of most suitable wavelength and Δ λ. It was found that λ= 311.8 nm and λ= 265.8 nm were suitable for analysis of sildenafil and paracetamol, respectively by first derivative method at Δλ= 4nm. Good regression parameters, linearity and correlation coefficient were obtained.

### *3.1.2. For sildenafil and metronidazole*

Several trials were done for selection of most suitable wavelength and Δλ. It was found that λ= 319.5 nm and λ= 291.8 nm were suitable for analysis of sildenafil and metronidazole, respectively by first derivative method at Δ λ= 4nm. Good regression parameters were achieved.

### *3.1.3. For sildenafil and glyburide*

Several trials were done for selection of most suitable wavelength and Δλ. It was found that λ= 328nm is suitable for analysis of sildenafil by direct spectrophotometry while λ= 316.3nm was suitable for determination of glyburide by ratio derivative spectrophotometry at Δλ= 4nm. Good regression parameters, linearity and correlation coefficient were obtained.

## 3.2. Sildenafil and paracetamol mixture

The overlay of zero order UV spectra of sildenafil and paracetamol **(Figure 2a)** showed considerable overlap so, both drugs was determined by first derivative method at different wavelengths by adjusting scaling factor at 10 and Δλ= 4nm, sildenafil was determined at λ= 311.8 nm where paracetamol was zero crossing, paracetamol was determined at λ= 265.8 nm where sildenafil was zero crossing **(Figure 3a)**. Calibration curves were constructed by plotting the amplitude of derivative values of absorbance against the corresponding concentration (µg/mL) for each of sildenafil and paracetamol. The regression equations were calculated with good correlation coefficient as shown in Table (1a).

## 3.3. Sildenafil and metronidazole mixture

The overlay of zero order UV spectra of sildenafil and metronidazole **(Figure 2b)** showed considerable overlap so, both drugs was determined with first derivative method at different wavelengths by adjusting scaling factor at 10 and Δ λ= 4nm. Sildenafil was determined at λ= 319.5 nm where metronidazole was zero crossing. Metronidazole was determined at λ= 291.8 nm where sildenafil was zero crossing **(Figure 3b)**. Calibration curves were obtained by plotting the amplitude of derivative values of absorbance against the corresponding concentration (µg/mL) for each of sildenafil and metronidazole. The regression equations were demonstrated with good correlation coefficient as shown in Table (1b).

## 3.4. Sildenafil and glyburide mixture

The overlay of zero order UV spectra of sildenafil and glyburide **(Figure 2c)** showed that sildenafil could be determined by direct spectrophotometric method at λ= 328nm where glyburide absorbance was zero. Glyburide was determined by ratio derivative method at λ= 316.3 nm using sildenafil 20µg/mL as divisor by adjusting scaling factor at 10 and Δ λ=4 nm **(Figure 3c)**. Calibration curves were constructed by plotting the absorbance values against the corresponding concentration (µg/mL) for sildenafil and plotting the amplitude of the derivative of ratio spectra against the corresponding concentrations (µg/mL) for glyburide. The regression equations were calculated with good correlation coefficient as shown in Table (1c). Each mixture was prepared three times and measured for accuracy studies as shown in Table (2).

## 3.5. Method validation

The proposed methods were validated as per ICH guidelines.[24]

 

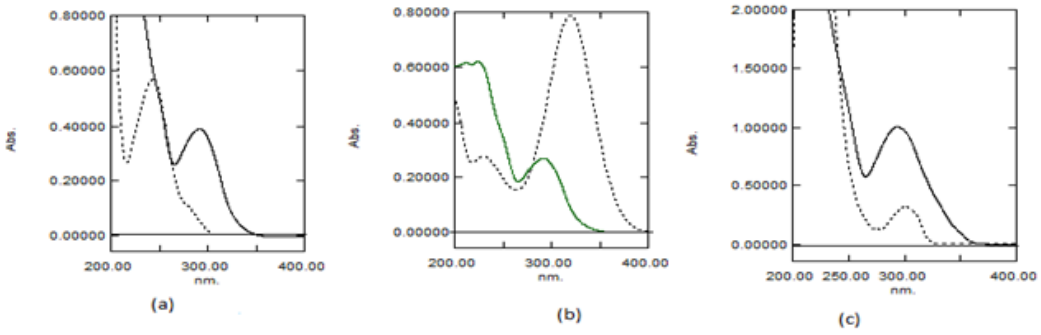


**Figure 2:** Overlay of zero order UV spectra of (a) 20 µg/mL sildenafil (-) and 9 µg/mL paracetamol (…) in distilled water, (b) 15 µg/mL sildenafil (-) and 15 µg/mL metronidazole (…) in distilled water, (c) 50 µg/mL sildenafil (-) and 50 µg/mL glyburide in ethanol.

**Table 1:** Regression parameters for determination of cited drugs by the proposed methods.

| Regression parameters | (a) Mixture of sildenafil and paracetamol | | (b) Mixture of sildenafil and metronidazole | | (c) Mixture of sildenafil and glyburide | |
|---|---|---|---|---|---|---|
| | Sildenafil | Paracetamol | Sildenafil | Metronidazole | Sildenafil | Glyburide |
| Linearity range (µg/mL) | 10-42 | 4-12.5 | 10-45 | 4-16 | 20-100 | 30-130 |
| $r^2$ | 0.9998 | 0.9998 | 0.9999 | 0.9999 | 0.9997 | 0.9999 |
| a | 0.0001 | 0.0040 | 0.0017 | 0.0026 | 0.0106 | -0.0006 |
| b | -0.0073 | -0.0174 | -0.0049 | -0.0114 | 0.0076 | -0.0137 |
| $s_a$ | 0.0013 | 0.0011 | 0.0007 | 0.0005 | 0.0036 | 0.0049 |
| $s_b$ | 4.46 x$10^{-5}$ | 1.3 x$10^{-5}$ | 2.52 x$10^{-5}$ | 5.12 x$10^{-5}$ | 5.76 x$10^{-5}$ | 5.93 x$10^{-5}$ |
| $S_{(y/x)}$ | 8.10 x$10^{-6}$ | 3.83 x$10^{-6}$ | 3.13 x$10^{-6}$ | 1.31 x$10^{-6}$ | 8.25 x$10^{-5}$ | 9.22 x$10^{-5}$ |
| LOD (µg/mL) | 0.189 | 0.036 | 0.111 | 0.038 | 0.152 | 0.016 |
| LOQ (µg/mL) | 0.572 | 0.11 | 0.335 | 0.115 | 0.460 | 0.049 |

r2: correlation coefficient, a: intercept, b slope, sa : standard deviation of intercept, sb: standard deviation of slope, S(y/x): residual standard deviation, LOD: limit of detection, LOQ: limit of quantitation.

### *3.5.1. Linearity*

Sildenafil has linear range within 10-42 µg/mL while paracetamol linearity was 4-12.5 µg/mL in sildenafil and paracetamol mixture as shown in Table (1a). In sildenafil and metronidazole mixture, the linearity range was 10-45 µg/mL and 4-16 µg/mL for sildenafil and metronidazole, respectively as shown in Table (1b). In sildenafil and glyburide mixture, the linearity range of sildenafil was 20-100 µg/mL while glyburide had linearity range of 30-130 µg/mL as shown in Table (1c). The corresponding regression equations for the cited drugs are shown in Table (1) with good correlation coefficient.

**Table 2:** Evaluation of accuracy for determination of cited drugs by the proposed methods.

| Mixture | Drug | Added conc. (µg/mL) | Found conc.* (µg/mL) | % Recovery | Mean % Recovery ± SD |
|---|---|---|---|---|---|
| **Sildenafil and paracetamol** | Sildenafil | 12 | 12.12 | 101.02 | 100.43 ± 0.60 |
| | | 12 | 11.98 | 99.82 | |
| | | 21 | 21.10 | 100.46 | |
| | Paracetamol | 8 | 8.03 | 100.39 | 100.28 ± 0.74 |
| | | 12 | 11.94 | 99.52 | |
| **Sildenafil and metronidazole** | Sildenafil | 9 | 9.09 | 100.99 | 101.50 ± 0.51 |
| | | 15 | 15.14 | 101.95 | |
| | | 15 | 15.14 | 100.95 | |
| | Metronidazole | 10 | 10.16 | 101.59 | 98.94 ± 0.31 |
| | | 10 | 9.86 | 98.62 | |
| | | 15 | 14.84 | 98.97 | |
| | | 15 | 14.89 | 99.24 | |
| **Sildenafil and glyburide** | Sildenafil | 40 | 40.44 | 101.10 | 100.13 ± 0.84 |
| | | 40 | 39.83 | 99.58 | |
| | Glyburide | 30 | 29.91 | 99.71 | 99.35 ± 1.22 |
| | | 40 | 39.51 | 98.78 | |
| | | 60 | 60.45 | 100.75 | |
| | | 70 | 68.96 | 98.511 | |

SD: standard deviation, * mean of three determinations

### *3.5.2. Accuracy*

Three mixtures for each combination were taken within linearity range and determined (triple). The values for mean % recovery ± SD shown in Table (2) indicate the accuracy of the proposed methods.

### *3.5.3. Limit of detection (LOD) & Limit of quantitation (LOQ)*

The values of LOD, LOQ for the cited drugs shown in Table (1) indicate the sensitivity of the proposed methods.

$$LOD = (3.3x\ \sigma) / b \quad \text{and} \quad LOQ = (10x\ \sigma) / b$$

where: σ is the standard deviation of response, and b is the slope of calibration curve.

### *3.5.4. Precision*

Three mixtures of each combination were prepared and determined (triple). For intraday precision the method was repeated three times within day and the average % RSD was determined as shown in Table (3). For inter day precision, the method was repeated on three different day and average % RSD was determined as shown in Table (4).

### *3.5.5. Selectivity*

The selectivity of the developed methods was indicated by the good mean % recovery ± SD for the cited drugs in presence of excipients using the proposed methods as well as by comparison between the spectrum of mixture of standard solution of cited drugs and that of laboratory prepared tablet regarding peak shape and position as shown in Figure (4).

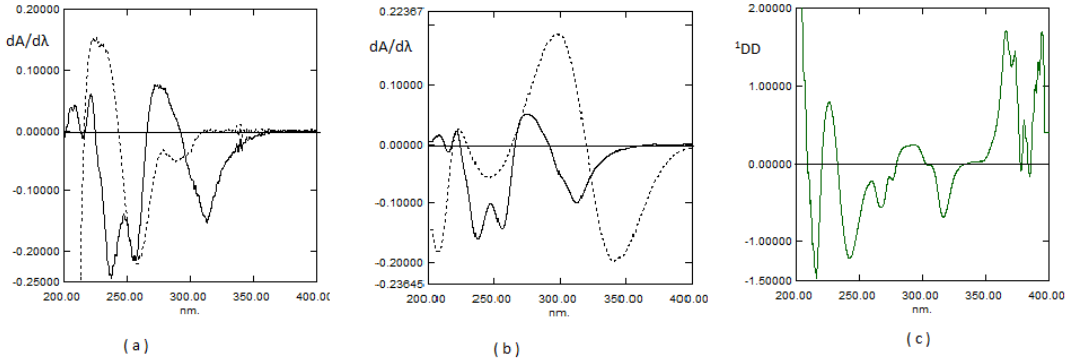


**Figure 3:** (a) Overlay of first derivative UV spectra of 20 µg/mL sildenafil (-) and 9 µg/mL paracetamol (…) in distilled water, (b) overlay of first derivative UV spectra of 15µg/mL sildenafil (-) and 15µg/mL metronidazole (…) in distilled water, (c) first derivative of the ratio spectrum of 50 µg/mL glyburide using 20 µg/mL sildenafil as divisor in ethanol.

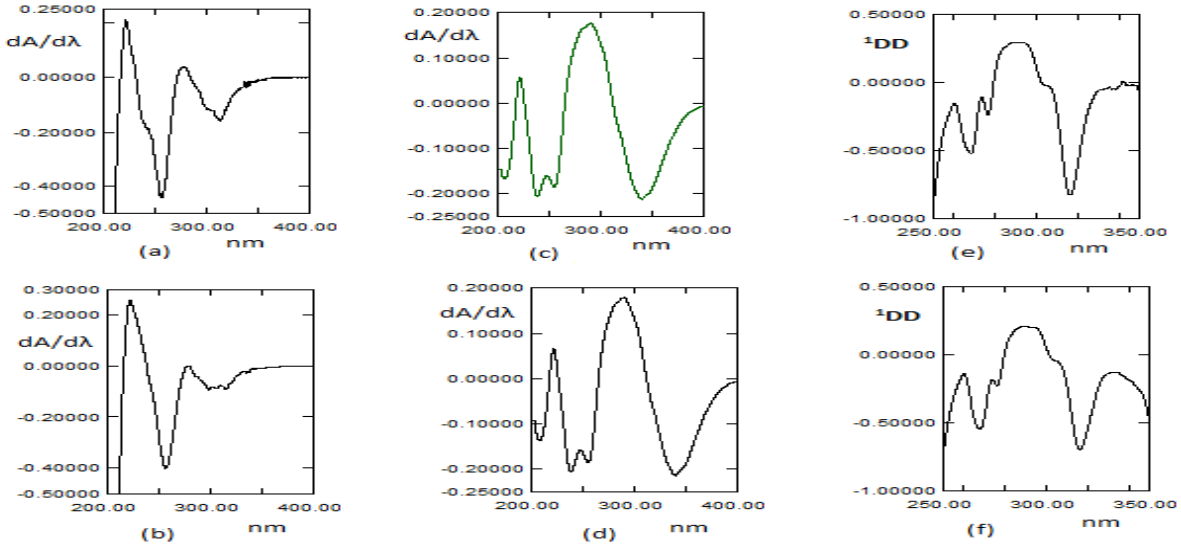


**Figure 4:** (a) First derivative spectrum of mixture of sildenafil 12 µg/mL and paracetamol 12 µg/mL, and (b) First derivative spectrum of its corresponding prepared tablet. (c) First derivative spectrum of mixture of sildenafil 15 µg/mL, metronidazole 15 µg/mL, and (d) First derivative spectrum of its corresponding prepared tablet. (e) First derivative of the ratio spectrum of glyburide 50 and sildenafil 50 mixture using sildenafil 20 µg/mL as divisor, and (f) First derivative of first derivative spectrum of its corresponding prepared tablet using sildenafil 20 µg/mL as divisor.

 

**Table 3:** Evaluation of intraday precision for determination of cited drugs by proposed methods.

**Mixture of sildenafil and paracetamol**

| | Day 1 | | | | | | Day 2 | | | | | | Day 3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sildenafil | | | Paracetamol | | | Sildenafil | | | Paracetamol | | | Sildenafil | | | Paracetamol | | |
| **Added C** | 12 | 12 | 21 | 8 | 12 | 9 | 12 | 12 | 21 | 8 | 12 | 9 | 12 | 12 | 21 | 8 | 12 | 9 |
| **Found C*** | 12.12 | 11.97 | 21.10 | 8.03 | 11.94 | 9.09 | 12.02 | 12.13 | 20.97 | 8.02 | 12.09 | 8.99 | 12.04 | 11.90 | 21.16 | 7.87 | 11.94 | 8.99 |
| **SD** | 0.06 | 0.01 | 0.16 | 0.04 | 0.096 | 0.04 | 0.07 | 0.13 | 0.32 | 0.05 | 0.097 | 0.12 | 0.11 | 0.09 | 0.26 | .039 | 0.13 | 0.02 |
| **% RSD** | 0.48 | 0.12 | 0.74 | 0.54 | 0.81 | 0.41 | 0.62 | 1.05 | 1.53 | 0.56 | 0.81 | 1.30 | 0.94 | 0.75 | 1.23 | 0.49 | 1.08 | 0.18 |

**Mixture of sildenafil and metronidazole**

| | Day 1 | | | | | | Day 2 | | | | | | Day 3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sildenafil | | | Metronidazole | | | Sildenafil | | | Metronidazole | | | Sildenafil | | | Metronidazole | | |
| **Added C** | 15 | 15 | 10 | 10 | 15 | 15 | 15 | 15 | 10 | 10 | 15 | 15 | 15 | 15 | 10 | 10 | 15 | 15 |
| **Found C*** | 15.14 | 15.14 | 10.16 | 9.86 | 14.85 | 14.89 | 15.22 | 14.95 | 10.04 | 9.99 | 14.90 | 14.83 | 14.99 | 15.17 | 10.08 | 9.90 | 14.78 | 14.78 |
| **SD** | 0.03 | 0.17 | 0.03 | 0.05 | 0.12 | 0.12 | 0.05 | 0.12 | 0.09 | 0.10 | 0.14 | 0.02 | 0.07 | 0.12 | 0.13 | 0.09 | 0.06 | 0.05 |
| **% RSD** | 0.19 | 1.13 | 0.28 | 0.51 | 0.78 | 0.79 | 0.34 | 0.83 | 0.87 | 0.985 | 0.97 | 0.15 | 0.49 | 0.77 | 1.25 | 0.86 | 0.43 | 0.35 |

**Mixture of sildenafil and glyburide**

| | Day 1 | | | | | | Day 2 | | | | | | Day 3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sildenafil | | | Glyburide | | | Sildenafil | | | Glyburide | | | Sildenafil | | | Glyburide | | |
| **Added C** | 40 | 40 | 30 | 40 | 60 | 70 | 40 | 40 | 30 | 40 | 60 | 70 | 40 | 40 | 30 | 40 | 60 | 70 |
| **Found C*** | 40.44 | 39.83 | 29.91 | 39.51 | 60.45 | 68.96 | 40.67 | 40.62 | 29.90 | 39.96 | 59.94 | 69.43 | 39.27 | 40.42 | 30.08 | 39.92 | 59.47 | 70.27 |
| **SD** | 0.08 | 0.19 | 0.22 | 0.14 | 0.39 | 0.14 | 0.12 | 0.12 | 0.17 | 0.57 | 0.39 | 0.95 | 0.05 | 0.24 | 0.18 | 0.01 | 0.64 | 0.27 |
| **% RSD** | 0.20 | 0.48 | 0.72 | 0.36 | 0.64 | 0.21 | 0.28 | 0.29 | 0.56 | 1.42 | 0.65 | 1.38 | 0.14 | 0.59 | 0.60 | 0.030 | 1.07 | 0.38 |

*: mean found concentration for 3 determinations, C: concentration (µg/mL), SD: standard deviation, %RSD: relative standard deviation.

**Table 4:** Evaluation of inter-day precision for determination of cited drugs by proposed methods.

| **Mixture of sildenafil and paracetamol** | | | | | | |
|---|---|---|---|---|---|---|
| | Sildenafil | | | Paracetamol | | |
| Added C | 12 | 12 | 21 | 8 | 12 | 9 |
| FC day1 | 12.12 | 11.98 | 21.10 | 8.03 | 11.94 | 9.09 |
| FC day2 | 12.02 | 12.13 | 20.96 | 8.02 | 12.09 | 8.99 |
| FC day3 | 12.04 | 11.89 | 21.16 | 7.87 | 11.94 | 8.99 |
| MFC | 12.06 | 12.00 | 21.07 | 7.97 | 11.99 | 9.03 |
| SD | 0.06 | 0.12 | 0.10 | 0.09 | 0.08 | 0.06 |
| % RSD | 0.47 | 0.99 | 0.47 | 1.08 | 0.70 | 0.61 |
| **Mixture of sildenafil and metronidazole** | | | | | | |
| | Sildenafil | | | Metronidazole | | |
| Added C | 15 | 15 | 10 | 10 | 15 | 15 |
| FC day1 | 15.14 | 15.14 | 10.16 | 9.86 | 14.85 | 14.89 |
| FC day2 | 15.22 | 14.95 | 10.04 | 9.99 | 14.90 | 14.83 |
| FC day3 | 14.99 | 15.17 | 10.08 | 9.90 | 14.78 | 14.78 |
| MFC | 15.12 | 15.09 | 10.09 | 9.92 | 14.84 | 14.83 |
| SD | 0.12 | 0.12 | 0.06 | 0.070 | 0.06 | 0.05 |
| % RSD | 0.77 | 0.80 | 0.62 | 0.70 | 0.42 | 0.36 |
| **Mixture of sildenafil and glyburide** | | | | | | |
| | Sildenafil | | | Glyburide | | |
| Added C | 40 | 40 | 30 | 40 | 60 | 70 |
| FC day1 | 40.64 | 40.68 | 30.25 | 39.16 | 60.01 | 70.49 |
| FC day2 | 40.27 | 40.62 | 29.79 | 40.13 | 59.93 | 69.45 |
| FC day3 | 39.31 | 40.45 | 30.10 | 39.91 | 59.49 | 70.25 |
| MFC | 40.07 | 40.58 | 30.044 | 39.73 | 59.81 | 70.06 |
| SD | 0.69 | 0.12 | 0.233 | 0.51 | 0.28 | 0.55 |
| % RSD | 1.71 | 0.30 | 0.776 | 1.27 | 0.46 | 0.78 |

C: concentration (µg/mL), FC: found concentration (µg/mL), MFC: mean found concentration (µg/mL), SD: standard deviation, RSD: relative standard deviation.

## 3.6. Assay of synthetic mixture

First derivative of laboratory prepared tablet of sildenafil with paracetamol or metronidazole showed good recovery from 98-102% and acceptable standard deviation not more than 2 for each drug that was measured triple as shown in Table (5 a, b). In tablet of sildenafil with glyburide, sildenafil was determined by direct spectrophotometry while glyburide was determined by first derivative of the ratio

spectrum. Good recovery (98-102%) was obtained with accepted standard deviation (not more than 2) as shown in Table (5c).

**Table 5:** Assay results for determination of cited drugs in their laboratory prepared tablets.

**(a) Tablet of sildenafil and paracetamol**

| Sample | % Recovery of sildenafil | % Recovery of paracetamol |
|---|---|---|
| 1 | 99.64 | 98.32 |
| 2 | 101.03 | 98.65 |
| 3 | 100.23 | 99.73 |
| Mean % recovery ± SD | 100.30 ± 0.70 | 98.90 ± 0.74 |

**(b) Tablet of sildenafil and metronidazole**

| Sample | % Recovery of sildenafil | %Recovery of metronidazole |
|---|---|---|
| 1 | 98.22 | 100.49 |
| 2 | 99.32 | 100.95 |
| 3 | 98.05 | 100.64 |
| Mean % recovery ± SD | 98.53 ± 0.69 | 100.69 ± 0.24 |

**(c) Tablet of sildenafil and glyburide**

| Sample | % Recovery of sildenafil | % Recovery of glyburide |
|---|---|---|
| 1 | 101.09 | 100.87 |
| 2 | 100.27 | 101.60 |
| 3 | 100.00 | 101.89 |
| Mean % recovery ± SD | 100.45 ± 0.57 | 101.46 ± 0.53 |

SD: standard deviation.

## 4. CONCLUSION

UV spectrophotometric methods were developed and validated for analysis of counterfeit sildenafil with paracetamol, metronidazole, and glyburide. The developed methods were evidenced to be simple, rapid, economic, accurate and precise as well as ecofriendly. The developed methods have superiority over HPLC methods for being simple, cost effective and time-saving. Thus it can be used for monotonous analysis of counterfeit sildenafil.

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