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Validated spectrophotometric methods for simultaneous determination of Omeprazole, Tinidazole and Doxycycline in their ternary mixture

Hayam M. Lotfy [a,b], Maha A. Hegazy [a], Shereen Mowaka [c,d], Ekram Hany Mohamed [c,*]

[a] Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, 11562 Cairo, Egypt
[b] Pharmaceutical chemistry department, Faculty of Pharmaceutical science and Pharmaceutical industries, Future University, 12311 Cairo, Egypt
[c] Analytical Chemistry Department, Faculty of Pharmacy, British University in Egypt, 11837 El-Sherouk City, Egypt
[d] Analytical Chemistry Department, Faculty of Pharmacy, Helwan University, Ein Helwan, 11795 Cairo, Egypt

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ABSTRACT

A comparative study of smart spectrophotometric techniques for the simultaneous determination of Omeprazole (OMP), Tinidazole (TIN) and Doxycycline (DOX) without prior separation steps is developed. These techniques consist of several consecutive steps utilizing zero/or ratio/or derivative spectra. The proposed techniques adopt nine simple different methods, namely direct spectrophotometry, dual wavelength, first derivative-zero crossing, amplitude factor, spectrum subtraction, ratio subtraction, derivative ratio-zero crossing, constant center, and successive derivative ratio method. The calibration graphs are linear over the concentration range of 1–20 μg/mL, 5–40 μg/mL and 2–30 μg/mL for OMP, TIN and DOX, respectively. These methods are tested by analyzing synthetic mixtures of the above drugs and successfully applied to commercial pharmaceutical preparation. The methods that are validated according to the ICH guidelines, accuracy, precision, and repeatability, were found to be within the acceptable limits.

1. Introduction

Peptic ulcers are painful sores in the lining of the stomach or the duodenum as a result of imbalance between digestive fluids, caused by infection with a type of bacteria called Helicobacter pylori or excess production of the acid producing cells of the stomach. For bacterial infection, the most effective treatment is a combination of 2 antibiotics (e.g. Tinidazole and Doxycycline) and 1 proton pump inhibitor (e.g. Omeprazole).

The three investigated drugs are officially listed in B.P. [1] and U.S.P. [2]; the chemical structures of the drugs are shown in Fig. 1.

Omeprazole (OMP) is a proton pump inhibitor, used in treatment of peptic ulcer disease and NSAID-associated ulceration, in gastro-oesophageal reflux disease and the Zollinger–Ellison syndrome [3]. OMP has been estimated as single or in combination with other drugs using several techniques including spectrophotometry [4,5], spectrofluorimetry [6], electrochemical methods [7], thin layer chromatography (TLC) [8,9], high performance liquid chromatography (HPLC) [10,11] and capillary electrophoresis [12].

Tinidazole (TIN) represents a class of drugs, namely anti-parasites that have an activity against anaerobic bacteria and protozoa. It is used in peptic ulcer with other antimicrobials and proton pump inhibitor [3]. TIN was determined as single or in combinations using different techniques including spectrophotometry [13–15], electrochemical methods [16], TLC [17] and HPLC [18–20].

Doxycycline (DOX) is a tetracycline derivative which is bacteriostatic with a broad spectrum of antimicrobial activity including many aerobic and anaerobic Gram-positive and Gram-negative pathogenic bacteria and some protozoa. It is used in triple therapy along with Tinidazole and proton pump inhibitor in the treatment of peptic ulcer [3].

Doxycycline had been estimated in pharmaceutical formulations by UV-spectrophotometry [21,22], spectrofluorimetry [23], electrochemical methods [24], TLC [25] HPLC [26,27] and capillary electrophoresis [28].

OMP was simultaneously determined in the presence of TIN using different techniques including spectrophotometry [5], electrochemical methods [29] TLC [8,9] and HPLC [30], while TIN was simultaneously determined in the presence of DOX using spectrophotometric technique [31].

Literature survey reveals that the three drugs were simultaneously determined using HPLC technique [32–34] while no reported methods have been reported for determination of the three drugs in combination using spectrophotometric methods.

The aim of this work was to develop spectrophotometric-methods based on smart original mathematical techniques for resolving the ternary mixture of OMP, TIN and DOX drugs with spectral interfering problems without preliminary separation. Consequently, we conduct a comparative study between the two recently developed methods namely; constant center (CC) [35], spectrum subtraction (SS) [36] and

* Corresponding author at: BUE el sherouk city, Egypt.
E-mail address: ekram.hany@bue.edu.eg (E.H. Mohamed).

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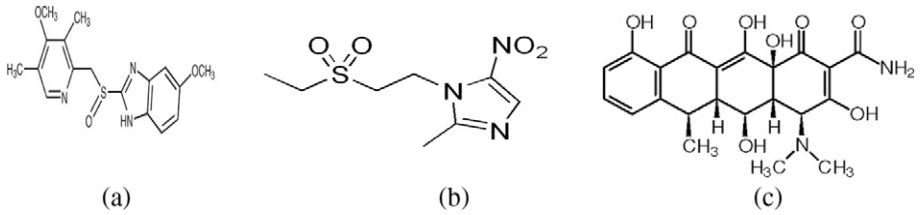

Fig. 1. Structural formulae for (a) Omeprazole, (b) Tinidazole, and (c) Doxycycline.

amplitude factor (P-Factor) [37–39] and conventional spectrophotometric methods namely; dual wavelength (DW) [40,41], ratio subtraction (RS) [42] first derivative (^{1}D) [43] derivative ratio-zero crossing (^{1}DD) [44] and successive ratio-derivative [45] in terms of specificity and validation and prove their effectiveness compared to the reported methods. The proposed methods are very simple, accurate, precise and do not require any sophisticated apparatus or computer programs.

For applying the proposed methods, only a spectrophotometer with simple software to measure and manipulate the spectra of the studied drugs is needed. At the same time the adopted methods are smart and can be applied for most of the binary and ternary mixtures with no need for any sophisticated conditions or difficult requirements. The only requirement for the dual wavelength method is the selection of two wavelengths for each drug in a way so that the difference in absorbance is zero for another drug. While for ratio subtraction, one of the drugs should be extended than the other one.

2. Experimental

2.1. Apparatus and software

Spectrophotometric measurements were carried out on JASCO V-630 BIO Double-beam UV–vis spectrophotometer (S/N C367961148), using 1.00 cm quartz cells. Scans were carried out in the range from 200 to 400 nm at 0.1 nm intervals. Spectra Manager II software was used.

2.2. Samples and solvents

2.2.1. Pure samples

Omeprazole (OMP), Tinidazole (TIN) and Doxycycline (DOX) were kindly supplied by El-Hikma Pharmaceutical Company, Cairo, Egypt; the purity was certified to be 100.10 ± 1.34, 100.88 ± 1.65 and 100.14 ± 1.12 according to the reported method for OMP and TIN [5] and official method [2] for DOX.

2.2.2. Market sample

TRIO capsules dosage form, labeled to contain 20 mg (OMP)/ 500 mg(TIN)/50 mg(DOX) was kindly supplied by El-Hikma Pharmaceutical Company.

2.2.3. Solvents

Solvents were of spectroscopic analytical grade; ethanol and water (Sigma Aldrich, Germany).

2.3. Standard solutions

Stock standard solution of each of OMP, TIN and DOX (1 mg/mL) in ethanol:water (90:10, v/v) is prepared.

The prepared solutions were found to be stable without any degradation when stored in dark glass wares in the refrigerator at 4 °C for 3 days.

Working standard solutions for OMP and DOX (50 μg/mL) and TIN (100 μg/mL) were prepared from stock solutions (1 mg/mL) by appropriate dilutions with ethanol:water (90:10, v/v).

2.4. Procedure

The zero-order absorption spectra (D^0) of 10 μg/mL for each of OMP, TIN and DOX were recorded in the range of 200–400 nm against ethanol:water (90:10, v/v) as a blank.

2.4.1. Construction of calibration graphs

Aliquots equivalent to 10–200 μg OMP, 50–400 μg TIN and 20–300 μg DOX were accurately transferred from their working standard solutions into three separate series of 10-mL volumetric flasks then completed to volume with the same solvent. The spectra of the prepared standard solutions were scanned from 200 to 400 nm and stored in the computer.

Calibration graphs were constructed relating the absorbance of zero order spectra (D^0) of OMP at 302 nm, TIN at 315 nm while DOX at 276 nm and 351 nm versus the corresponding concentrations.

For dual wavelength method calibration graphs relating the difference between the absorbance at 287.2 nm and 263.2 nm for OMP and that between 292.7 nm and 309.6 nm for DOX versus the corresponding concentrations and the regression equations were computed.

Then the first derivative spectra (D^1) ($\Delta\lambda = 8.00$ and scaling factor = 10) were recorded and calibration graphs were constructed relating the amplitude of the obtained (D^1) spectra of OMP, TIN, and DOX at 313 nm, 352 nm and 380 nm respectively versus the corresponding concentrations and the regression equations were computed. A factor (P_{380}/P_{352}) relating the amplitude of TIN at 380 nm and 352 nm was calculated to be 0.146.

For constant center method the stored absorption spectra of TIN and DOX were divided by the absorption spectra of 5 μg/mL DOX and 5 μg/mL TIN, respectively where the obtained ratio spectra were recorded. Calibration graphs were constructed by plotting the difference between the amplitudes of the obtained ratio spectra at [350 nm and 360 nm] and [361 nm and 374 nm]; versus amplitudes at 350 nm and 374 nm for TIN and DOX respectively and the regression equations were computed.

For derivative ratio-zero crossing method the stored (D^0) spectra of OMP were divided by the spectrum of 5 μg/mL DOX and the first derivative of the obtained spectra is recorded. A calibration graph relating the amplitude at 313.9 nm versus the corresponding concentrations of OMP was constructed and the regression equations were computed.

For successive ratio-derivative spectra, the stored zero order absorption spectra (D^0) of different concentration of OMP were divided by the spectrum of 5 μg/mL of DOX and the ratio spectra were obtained. First derivatives of the ratio spectra were obtained with $\Delta\lambda = 8$ and scaling factor 1. These vectors (D^1 of the ratio spectra) were divided by (d/dλ) (5 μg/mL of TIN / 5 μg/mL of DOX) corresponding to the derivative of the ratio of the spectra of TIN and DOX and therefore, second ratio spectra were obtained. First derivative of these vectors was obtained $\Delta\lambda = 8$ and the calibration graph of OMP was constructed by plotting the amplitude of the resulting spectra at 314.5 nm against its corresponding concentration. The same steps were performed for TIN using the spectrum of 5 μg/mL of DOX as the first divisor followed by the spectrum of (d/dλ) (5 μg/mL of OMP / 5 μg/mL of DOX) as the second divisor; and for DOX using the spectrum of 5 μg/mL of TIN as