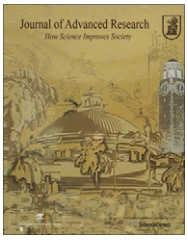
Journal of Advanced Research (2010) 1, 323–329

Cairo University

Journal of Advanced Research



ORIGINAL ARTICLE

Spectrophotometric methods for the simultaneous determination of binary mixture of metronidazole and diloxanide furoate without prior separation

Mohamed R. El-Ghobashy [a,*], **Nisreen F. Abo-Talib** [b]

[a] *Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Egypt*
[b] *National Organization for Drug Control and Research (NODCAR), Cairo, Egypt*

Received 26 October 2009; revised 1 March 2010; accepted 18 March 2010
Available online 30 June 2010

KEYWORDS

Metronidazole;
Diloxanide furoate;
Binary mixture;
Isosbestic point;
Ratio subtraction

Abstract Ratio subtraction and isosbestic point methods are two innovative spectrophotometric methods for determining the concentrations of metronidazole (I) and diloxanide furoate (II) in a mixture. Metronidazole was determined by direct spectrophotometric method at λ_{max} 314.0 nm in the presence of diloxanide furoate in the range of 4–24 μg ml^{-1} with a mean recovery percentage of 99.83 ± 1.41. Two spectrophotometric methods were developed for the spectral resolution of diloxanide furoate when present in mixture with metronidazole without preliminary separation. The first method depends on measuring the absorbance at the isosbestic point at 277.2 nm in the range of 5–30 μg ml^{-1} with a mean recovery percentage of 99.96 ± 1.47 for diloxanide furoate. The second method is the ratio subtraction spectroscopic method for spectral isolation of diloxanide furoate present in the mixture which can be measured at 251.2 nm in the range of 5–30 μg ml^{-1} with a mean recovery percentage of 99.73 ± 1.33 for diloxanide furoate determination. The suggested procedures were validated using laboratory-prepared mixtures and were successfully applied for the analysis of pharmaceutical preparations. The methods retained their accuracy and precision when the standard addition technique was applied. The results obtained by applying the proposed methods were statistically analyzed and compared with those obtained by the reported method.

* Corresponding author. Tel.: +202 33020604.
E-mail address: mohamedrefaat73@yahoo.com (M.R. El-Ghobashy).

Peer review under responsibility of Cairo University.
doi:10.1016/j.jare.2010.06.001

Production and hosting by Elsevier

Introduction

Metronidazole and diloxanide furoate are formulated together to be highly effective in the treatment of intestinal and extraintestinal amoebic infections. Metronidazole is less effective against parasites in the bowel lumen and is, therefore, used in combination with a luminal amoebicide, such as diloxanide furoate in the treatment of invasive amoebiasis.

Metronidazole 2-methyl-5-nitroimidazole-1-ethanol [1] was determined individually by short-wave length NIR

spectroscopy [2], voltammetry [3–5], NMR spectrometry [6], gas chromatography [7] and HPLC methods either alone [8–10] or in the presence of its metabolites [11,12] or in the presence of its degradation product [13], in addition to its mixture with other drugs [14,15]. First derivative spectrophotometry was used for the determination of metronidazole in mixture with ciprofloxacin [16].

Diloxanide furoate 2,2-dichloro-N-(4-hydroxyphenyl)-N-methylacetamide [1] was determined individually by colorimetric method [17], in the presence of its degradation product by derivative technique, derivative ratio, TLC-densitometry [18] and HPLC [18,19] and in mixture with tinidazole and furazolidone by second derivative spectrophotometry [20].

The main problem of spectrophotometric binary mixture analysis is the simultaneous determination of the two compounds in the same mixture without prior separation. One spectrophotometric determination method has been used for resolving such mixture with overlapping spectra, derivative spectrophotometry [21] and HPLC [22].

The aim of this work is to develop new spectrophotometric methods for resolving this mixture with spectral interfering problems, without preliminary separation. The new methods were very simple, did not require any computer programs (derivative and derivative ratio) as metronidazole was determined by direct spectrophotometry and diloxanide furoate was determined by simple mathematical calculation. Also the method used did not require any sophisticated instrumentation, such as HPLC, which requires solvents and time.

Experimental

Apparatus

Spectrophotometer: SHIMADZU UV-1601 PC, dual beam UV–visible spectrophotometer with two matched 1 cm quartz cells, connected to an IBM compatible personal computer (PC) and an HP-600 inkjet printer. Bundled UV-PC personal spectroscopy software version (3.7) was used to process the absorption and the derivative spectra. The spectral band width was 0.2 nm with wavelength scanning speed of 2800 nm min^{-1}.

Materials

Pure samples

Metronidazole and diloxanide furoate were kindly supplied by Egyptian Int. Pharmaceutical Industries Co., E.I.P.I.CO. 10th of Ramadan City, Area B1 P.O. 149, Egypt. Their purity was found to be 99.84 ± 1.26 and 100.50 ± 0.71, respectively, according to the manufacturer's direct spectrophotometric method (personal communication).

Market samples

Furazole tablets (E.I.P.I.CO.); batch no. 080435. It was labeled to contain 200 and 250 mg metronidazole and diloxanide furoate, respectively, per tablet.

Furazole suspension (E.I.P.I.CO.); batch no. 074135. It was labeled to contain 200 and 100 mg metronidazole and diloxanide furoate, respectively, per 5 ml.

Chemicals and reagents

All chemicals were of analytical grade and the solvents were of spectroscopic grade. Methanol, (E-Merck, Darmstadt, Germany).

Standard solutions

Stock solutions

Metronidazole (I) and diloxanide furoate (II) stock solutions (1 mg ml^{-1}) were prepared by weighing accurately 100 mg of each powder into two separate 100 ml volumetric flasks. Methanol (50 ml) was added, shaken for a few minutes and completed to volume with the same solvent.

Working solutions

Four micro litres of the stock solution of (I) and 5 ml of the stock solution (II) were accurately transferred into two separate 50 ml measuring flasks and diluted to the mark with methanol to get a final concentration of 80 μg ml^{-1} and 100 μg ml^{-1} of (I) and (II), respectively.

Laboratory-prepared mixtures

Accurate aliquots equivalent to (40–100 μg) of (I) were transferred from its working solution (80 μg ml^{-1}) into a series of 10 ml volumetric flasks and portions equivalent to (50–150 μg) of (II) from its working solution (100 μg ml^{-1}) were added to the same flasks and volumes were completed to mark with methanol and mixed well.

Procedures

Isosbestic spectrophotometric method

Linearity: aliquots from (I) and (II) working solutions (80 μg ml^{-1} of (I) and 100 μg ml^{-1} of (II), respectively) equivalent to 40–240 μg of (I) and 50–300 μg of (II) were transferred into two separate sets of 10 ml volumetric flasks and completed to the mark with methanol. The zero order absorption spectra were recorded for both drugs using methanol as a blank; then the absorbance was measured at 314.0 nm for (I) and 277.2 nm (A_{iso}) for (I) and (II). Two calibration curves were constructed for each drug relating the absorbance at the selected wavelength to the corresponding drug concentrations and the regression equations were computed.

Assay of laboratory-prepared mixtures: Absorbance of the spectra of laboratory-prepared mixtures containing different ratios of (I) and (II) were measured at 314.0 nm corresponding to the contents of (I) only, and at 277.2 nm (A_{iso}) corresponding to the total content of (I) and (II) in the mixture. The concentration of (I) alone and the total concentration of the two drugs were calculated from their corresponding regression equations; then by subtraction of (I) concentration from the total mixture concentration, yielding the actual concentration of (II) in the mixture.

Ratio subtraction spectrophotometric method

Linearity: Aliquots containing 50–300 μg from (II) working solution (80 μg ml^{-1}) were transferred into a series of 10 ml volumetric flasks then completed to volume with methanol;