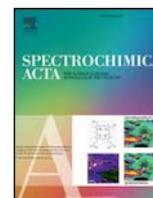




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Comparative study of the efficiency of computed univariate and multivariate methods for the estimation of the binary mixture of clotrimazole and dexamethasone using two different spectral regions

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ABSTRACT

Three methods of analysis are conducted that need computational procedures by the Matlab® software. The first is the univariate mean centering method which eliminates the interfering signal of the one component at a selected wave length leaving the amplitude measured to represent the component of interest only. The other two multivariate methods named PLS and PCR depend on a large number of variables that lead to extraction of the maximum amount of information required to determine the component of interest in the presence of the other. Good accurate and precise results are obtained from the three methods for determining clotrimazole in the linearity range 1–12 µg/mL and 75–550 µg/mL with dexamethasone acetate 2–20 µg/mL in synthetic mixtures and pharmaceutical formulation using two different spectral regions 205–240 nm and 233–278 nm. The results obtained are compared statistically to each other and to the official methods.

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1. Introduction

Clotrimazole (CLT) [1 [(2 Chlorophenyl)diphenylmethyl] 1H imidazole] is an antifungal agent that leads to cell death of fungal cells by inhibiting ergosterol biosynthesis [1]. BP encloses a titrimetric method for determining CLT [2] and an HPLC method in the USP [3]. The literature revealed also colorimetric methods [4–6], spectrophotometric methods [7, 8] and HPLC methods [4, 9–11] for quantitative estimation of CLT.

The corticosteroid dexamethasone acetate (DA) [16 methyl 11β, 17α, 21 trihydroxy 9α fluoropregna 1,4 diene 3,20 dione 21 acetate] is an anti-inflammatory drug [12]. USP describes an HPLC method for determining DA [3], while in the BP it is determined by direct spectrophotometry [2]. The literature revealed also determination of DA by HPLC [13–15], Chemometrics-assisted spectrophotometric-assisted spectrophotometry [16], and capillary electrophoresis [17].

There is an established RP-HPLC method that can analyze both DA and CLT together in mixtures [18], and several conventional and mathematically manipulated spectrophotometric methods [19].

Chemical structures are illustrated in Fig. 1.

The aim of this study is investigating the ability of the univariate mean centering (MCR) method and the multivariate chemometric approaches (PLS and PCR) to quantify the binary mixture of clotrimazole (CLT) and dexamethasone (DA) over a wide linearity range of concentrations, as their spectra are severely overlapped when CLT is in the concentration range 75–550 µg/mL in the wavelength region 233–278 nm, while CLT shows no sharp λ_{\max} in the concentration range 1–12 µg/mL, where it becomes partially overlapped with DA in the wavelength region 205–240 nm. Work is conducted without any prior separation and can be used in the analysis of their market formula named Mycuten-D® cream. Comparative study between the obtained results using the two spectral regions is applied to check the efficiency of the proposed methods in the estimation of the cited drugs.

2. Experimental

2.1. Instrument

A double-beam UV/Visible spectrophotometer model J-760, Jasco, Japan, connected to ACER compatible computer.

Software: Spectramanager® software, Matlab® version 7.9, and PLS-Toolbox 2.0.

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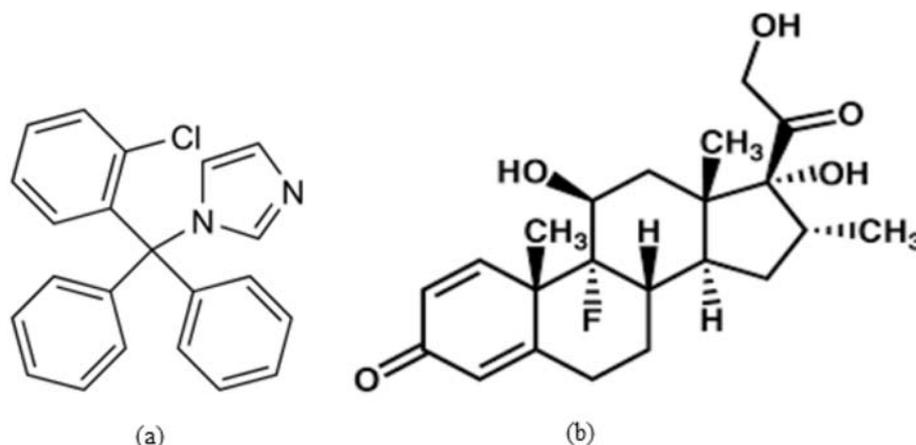


Fig. 1. The chemical structure of (a) clotrimazole, (b) dexamethasone acetate.

2.2. Chemical Reagents

2.2.1. Pure Samples

CLT supplied by Alexandria Company for Pharmaceuticals with purity 100.06 ± 0.16 , according to the USP [3], DA supplied by Sanofi Aventis with purity 99.99 ± 0.52 according to the BP [2].

2.2.2. Market Samples

Mycuten-D® cream each 1 g contains 10 mg CLT and 0.445 mg DA, manufactured by Alexandria company for pharmaceuticals and purchased from the Egyptian market.

2.2.3. Solvents

HPLC-grade anhydrous methanol is used manufactured by Carlo Erba Reagents.

2.3. Standard Solutions

- CLT standard stock solution; 1000 $\mu\text{g}/\text{mL}$ in methanol.
- DA standard stock solution; 500 $\mu\text{g}/\text{mL}$ in methanol.
- CLT and DA working solution; 50 $\mu\text{g}/\text{mL}$ in methanol.

2.4. Procedure

2.4.1. Spectral Data for CLT and DA

D^0 absorption spectra of CLT in two different concentrations (10 $\mu\text{g}/\text{mL}$ and 200 $\mu\text{g}/\text{mL}$) that show two totally different spectra. And the DA D^0 absorption spectra of (10 $\mu\text{g}/\text{mL}$), scanned against methanol as a blank (205–400 nm), overlaid using the Spectramanager software (Fig. 2) to detect the spectral characteristics, overlap, and predict the best methods for resolution and determination of concentration.

2.4.2. Construction of Calibration Curves in MCR

Two sets are prepared, the first one (Set A) contain CLT in low concentrations, while the second one (Set B) contains CLT in high concentrations.

Set A is a series of working standard solutions containing 1–12 $\mu\text{g}/\text{mL}$ CLT and 2–20 $\mu\text{g}/\text{mL}$ DA, that are prepared separately in methanol using their corresponding standard solutions (50 $\mu\text{g}/\text{mL}$). Set B is another series of working standard solutions containing 2–20 $\mu\text{g}/\text{mL}$ DA using its corresponding working standard solution (50 $\mu\text{g}/\text{mL}$), and 75–550 CLT using its corresponding standard stock solution (1000 $\mu\text{g}/\text{mL}$) prepared separately in methanol. The absorption spectra of the resulting

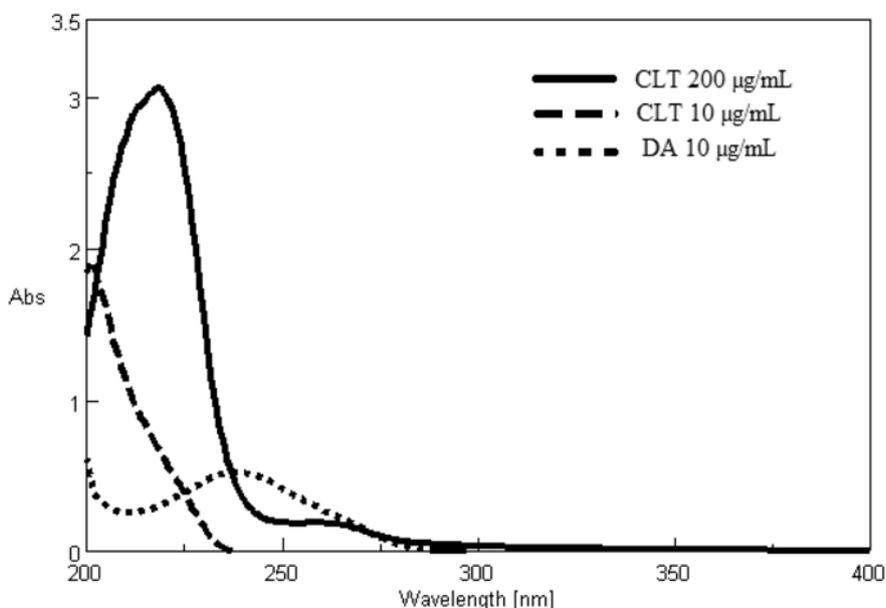


Fig. 2. zero order absorption spectra 10 $\mu\text{g}/\text{mL}$ dexamethasone (.....) overlaid with 200 $\mu\text{g}/\text{mL}$ clotrimazole (—) and 10 $\mu\text{g}/\text{mL}$ clotrimazole (-----).

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