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ORIGINAL ARTICLE

New simple spectrophotometric method for determination of the antiviral mixture of emtricitabine and tenofovir disoproxil fumarate

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Abstract A new simple spectrophotometric method was recently developed for the determination of binary mixtures without prior separation. The method is based on generation of ratio spectra of compound X by using standard spectrum of compound Y as a divisor, then the peak to peak (or peak to trough) amplitudes between two selected wavelengths in the ratio spectra are found proportional to concentration of X without interference from compound Y. The method was applied for the determination of the antiviral mixture of emtricitabine (EMT) and tenofovir disoproxil fumarate (TEN). For the determination of EMT, a solution of 15 μg/mL TEN was used as divisor to generate the ratio spectra, and the peak to trough amplitudes between 228.5 and 260.5 nm were plotted against EMT concentration. Similarly by using 10 μg/mL EMT as divisor, the peak to trough amplitudes between 229 and 260 nm were found proportional to TEN concentration. The reliability and analytical performance of the proposed spectrophotometric procedure were statistically validated with respect to linearity, range, precision, accuracy, detection and quantification limits. Calibration curves were linear in the range of 2–40 μg/mL for both drugs with correlation coefficients >0.9997. The proposed method was successfully applied for the simultaneous determination of the two drugs in several laboratory prepared mixtures and in their combined tablet dosage form.

1. Introduction

One of the main challenges facing analytical chemists is the spectrophotometric determination of two or more compounds in the same sample without preliminary separation. Several spectrophotometric methods have been used for resolving mixtures of compounds with overlapping spectra such as derivative spectrophotometry (Wahbi and Ebel, 1974; Morelli, 1994) Vierordt's method (Görög, 1995), dual wavelength

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spectrophotometry (Shibata et al., 1971), pH-induced difference spectrophotometry (Davidson and Stenlake, 1974), H-point standard addition method (HPSAM) (Sabry and Khamis, 2000) and several more sophisticated computerized and chemometric spectrophotometric methods (Korany et al.,1986, 1990; Wahbi et al., 1989; El-Gindy et al., 2010).

Generation of absorbance ratio spectra has been the basis of several analytical procedures for the simultaneous spectrophotometric determination of compounds in binary and ternary mixtures. First, binary mixtures were resolved using the ratio-spectra derivative method (Salinas et al., 1990). Then, this method was modified and extended for the determination of ternary mixtures. Examples of these modified versions are the derivative ratio spectra zero-crossing method (Berzas Nevado et al., 1992; Abdel-Hay et al., 2008), the double divisor ratio spectra derivative method (Dinç and Onur, 1998) and the successive derivative ratio spectra method (Afkhami and Bahram, 2005). Furthermore, the ratio spectra were exploited for the development of other mathematical methods for resolution of binary and ternary mixtures such as the mean centering of ratio spectra method (Abdelwahab et al., 2012), the ratio subtraction method (El-Bardicy et al., 2008) and the convoluted double divisor ratio spectra using combined trigonometric Fourier functions method (Youssef and Maher, 2008). In most of these methods, at least two mathematical processes (e.g. division followed by derivative curve generation) are needed in order to get measurable amplitude that is correlated to the concentration of only one compound without interference from the others in the mixture. Obviously, some methods need more sophisticated mathematical treatment to unambiguously determine the target compound in the presence of interferences (El-Bardicy et al., 2008; Youssef and Maher, 2008).

Emtricitabine (EMT) and tenofovir disoproxil fumarate (TEN) are reverse transcriptase inhibitors with antiviral activity against HIV-1 and hepatitis B virus. Fixed dose combination products of the two antiviral drugs for the treatment of HIV infection have been developed in order to improve patient adherence and avoid monotherapy, thereby decreasing the risk of acquired drug resistance (Sweetman, 2009; Truvada webpage). Several HPLC methods were found in the literature for the assay of this mixture in human plasma or in tablet dosage forms. Detection of both drugs in these methods was achieved either depending on UV-detection (Karunakaran et al., 2010; Pendela et al., 2011) or tandem mass spectrometry (Gomes et al., 2008; Delahunty et al., 2009; Yadav et al., 2010). HPTLC was also applied for the determination of this binary mixture (Joshi et al., 2009; Bhirud and Hiremath, 2013). Finally, few spectrophotometric methods were published for the simultaneous estimation of EMT and TEN in their combined tablets. These reports recommended the use of area under the curve and dual wavelength methods (Ghorpade et al., 2010), simultaneous equations and absorbance ratio methods (Ingale et al., 2010) and the absorbance correction method and first order derivative spectrophotometry (Karunakaran et al., 2011).

Recently, a new *ratio spectra peak to peak measurement method* was developed for the determination of binary mixtures without prior separation (Belal et al., 2013). The method employs only one step (dividing the mixture spectra by a standard divisor spectrum), followed by *peak to peak measurement* in the produced ratio spectra. The method eliminates the derivative step, and does not require searching for zero-crossing points or any complicated mathematical or chemometric treatment of data. This work describes the application of this new method for the quantification of the binary mixture of EMT and TEN where the two compounds were simultaneously determined in several laboratory prepared mixtures and in tablets without prior separation.

2. Theoretical background

Consider a mixture of two compounds X and Y. The absorption spectrum of the mixture-measured in 1 cm pathlength- is defined by the equation:

$$A_M = \epsilon_X C_X + \epsilon_Y C_Y \quad (1)$$

where A_M is the absorbance of the mixture, ε_X and ε_Y are the molar absorptivities of X and Y and C_X and C_Y are the concentrations of X and Y. If the absorbance of the mixture is divided by the absorbance of a standard solution of X (Absorbance $A^o_X = \varepsilon_X C^o_X$), the following equation results:

$$\frac{A_M}{A^o_X} = \frac{C_X}{C^o_X} + \frac{A_Y}{A_{X^o}} \quad (2)$$

The ratio C_X/C^0_X is a constant value which can be eliminated by taking the difference in absorbance ratio amplitudes between two wavelengths λ_1 and λ_2 (peak to peak measurement):

$$\left[\frac{A_M}{A^o_X}\right]_{\lambda 1} - \left[\frac{A_M}{A^o_X}\right]_{\lambda 2} = \left[\frac{A_Y}{A_{X^o}}\right]_{\lambda 1} - \left[\frac{A_Y}{A_{X^o}}\right]_{\lambda 2} \quad (3)$$

Eq. (3) illustrates that the difference amplitude in the mixture absorbance ratio between two wavelengths λ_1 and λ_2 (can be called *peak to peak*, *peak to trough* or *maximum to minimum* measurement) is equal to the same difference amplitude for compound Y after canceling the constant interference due to compound X. In the proposed method, the concentration of compound Y (C_Y) is proportional to the *peak to peak* amplitudes of its absorbance spectra. A calibration graph is obtained by recording and storing the spectra of solutions of different concentrations of pure Y, and the spectrum of a solution of pure X (the divisor X°). The stored spectra of the solutions of pure Y are divided by the standard spectrum of the divisor (X°). In the generated ratio spectra, the *peak to peak* amplitudes between the selected wavelengths λ_1 and λ_2 are measured and plotted against C_Y to obtain the calibration graph. By using the calibration graph, the concentration of compound Y in the mixture is determined after similar treatment for the mixture solution. The concentration of X in the mixture is determined by an analogous procedure.

3. Experimental

3.1. Apparatus

Spectrophotometric measurements were performed using a Specord S600 UV/VIS diode array spectrophotometer (scan speed 6000 nm/min and wavelength interval 0.5 nm), associated with WinAspect software version 2.3 (Analytik Jena AG, Germany).

3.2. Materials

Authentic samples of emtricitabine (EMT) and tenofovir disoproxil fumarate (TEN) were kindly provided by Gilead

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