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Comparative study of six sequential spectrophotometric methods for quantification and separation of ribavirin, sofosbuvir and daclatasvir: An application on Laboratory prepared mixture, pharmaceutical preparations, spiked human urine, spiked human plasma, and dissolution test

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ABSTRACT

In accordance with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines, six novel, simple and precise sequential spectrophotometric methods were developed and validated for the simultaneous analysis of Ribavirin (RIB), Sofosbuvir (SOF), and Daclatasvir (DAC) in their mixture without prior separation steps. These drugs are described as co-administered for treatment of Hepatitis C virus (HCV). HCV is the cause of hepatitis C and some cancers such as liver cancer (hepatocellular carcinoma) and lymphomas in humans. These techniques consisted of several sequential steps using zero, ratio and/or derivative spectra. DAC was first determined through direct spectrophotometry at 313.7 nm without any interference of the other two drugs while RIB and SOF can be determined after ratio subtraction through five methods; Ratio difference spectrophotometric method, successive derivative ratio method, constant center, isoabsorptive method at 238.8 nm, and mean centering of the ratio spectra (MCR) at 224 nm and 258 nm for RIB and SOF, respectively. The calibration curve is linear over the concentration ranges of (6–42), (10–70) and (4–16) μg/mL for RIB, SOF, and DAC, respectively. This method was successfully applied to commercial pharmaceutical preparation of the drugs, spiked human urine, and spiked human plasma. The above methods are very simple methods that were developed for the simultaneous determination of binary and ternary mixtures and so enhance signal-to-noise ratio. The method has been successfully applied to the simultaneous analysis of RIB, SOF, and DAC in laboratory prepared mixtures. The obtained results are statistically compared with those obtained by the official or reported methods, showing no significant difference with respect to accuracy and precision at $p = 0.05$.

1. Introduction

Hepatitis C is an infection caused by the hepatitis C virus (HCV) that attacks the liver and leads to inflammation. The World Health Organization (WHO) estimates about 71 million people globally have chronic hepatitis C, with approximately 399,000 dying from this infection as primarily due to cirrhosis and hepatocellular carcinoma [1].

Ribavirin (Fig. 1. a),1-β-D-ribofuranosyl-1H-1, 2, 4triazole-3-carboxamide [2], a nucleoside (purine analouge) antimetabolite broad spectrum antiviral agent that blocks nucleic acid synthesis and used against both RNA and DNA viruses. Ribavirin used for treating the infection of respiratory syncytial virus (RSV) in children and in combination with DAC and SOF for treatment of chronic hepatic virus C infection. Its action appeared through inhibition to the replication to a variety of RNA and DNA viruses [2]. After deeply literature investigation, it was found that several methods used for determination of ribavirin including spectrophotometry [3,4], liquid chromatography tandem mass spectrometry for the bioanalysis of Ribavirin either alone or in various combinations in biological matrices [5–13], and few RP-HPLC methods for the determination of ribavirin [14–16].

Chemically; daclatasvir dihydrochloride is methyl((1*S*)- 1-(((2*S*)-2-(5-(4′-(2-((2*S*)-1-((2*S*)-methoxycarbony)amino)-3- methylbutanoyl)-2-pyrrolidinyl)-1H-imidazol-5-yl)-4-biphenylyl)-1H-imidazol-2-yl)-1-pyrrolidinyl)carbonyl)-2-methylpropyl) carbamate dihydrochloride [17] (Fig. 1 b). DAC used for treatment of hepatitis-C virus (HCV) infection [18] through binding to HCV protein NS5A and so inhibition to its function [18]. From the literature survey, it is obvious that few methods

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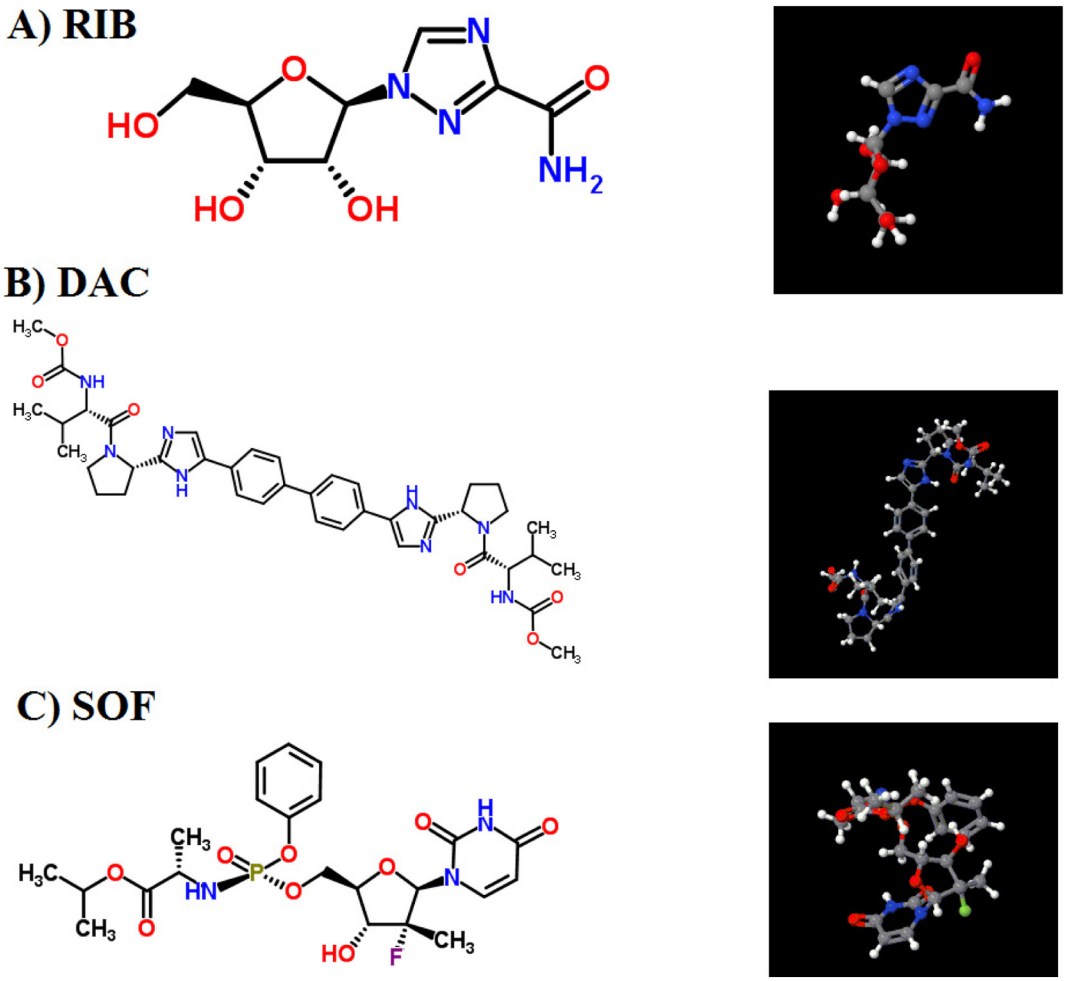

Fig. 1. Chemical and molecular structure of a) RIB, b) DAC, and c) SOF.

are available for DAC such as spectroscopic methods [19], HPLC-UV methods [20,21], RP-HPLC methods [22], LC-MS/MS methods [23,24].

Sofosbuvir, isopropyl(2*S*)-2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3- hydroxyl-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino] propanoate, is a prodrug (nucleotide analogue) used for treatment of hepatitis C virus (HCV) [25]. SOF is metabolized to the analog uridine triphosphate. This metabolite is pharmacologically active and can be joined to the ribonucleic acid (RNA) of the HCV leading to chain termination and so prevention to replication of the virus [26]. SOF can be used alone or co-administered with others like ribavarin and daclatasvir [27]. Co-administration of SOF with other drugs will result in increasing their activity against HCV virus. There is not many reported literature review for the estimation of SOF either alone or in combination with others as spectrophotometry [28], RP-HPLC methods [29–31], and LC-MS/MS methods [32,33]. Since there were no methods reported for the analysis of these three drugs in combination. The aim of this work was to develop simple, accurate and precise spectrophotometric methods based on smart original mathematical techniques for resolving the ternary mixture of RIB, DAC, and SOF in pure, laboratory prepared mixtures, pharmaceutical preparations, spiked human urine and spiked human plasma without prior separation (Scheme 1). Consequently, we conduct a comparative study between six sequential methods; Ratio difference spectrophotometric method, successive derivative ratio method, constant center, isoabsorptive method and mean centering of the ratio spectra (MCR) in terms of specificity and validation and prove their effectiveness compared to the reported methods. This work investigated a superior plan for simultaneous determination of these drugs in spiked human urine and spiked human plasma. Fast resolution to a complex system was obtained using univariate calibration techniques on spectral data.

2. Theoretical Backgrounds

2.1. Direct Spectrophotometry After Ratio Subtraction Method [34]

If you have a mixture of RIB, DAC, and SOF represented by X, Y (A) and Z, respectively with overlapping spectra and the spectrum of Z is extended more than A. Z can be determined directly at its λ_{max} so, a binary mixture of X and Y (A) was obtained that can be determined by ratio subtraction method. This method depends on dividing the spectrum of the mixture by a concentration of Z as a divisor (Z^0). The division will give a new curve that is shown in Eq. (1)

$$\frac{A+z}{z^0}=\frac{A}{z^0}+\frac{z}{z^0}=\frac{A}{z^0}+\text{constant} \quad (1)$$

If we subtract this constant (The constant can be determined directly from the curve $(A+Z)/Z^0$by the straight line which is parallel to the wavelength axis in the region where (Z) is extended). This can be illustrated in Eq. (2).

$$\frac{A}{z^0}+\text{constant}-\text{constant}=\frac{A}{z^0} \quad (2)$$