Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 126 (2014) 112–121

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

A comparative study of novel spectrophotometric methods based on isosbestic points; application on a pharmaceutical ternary mixture

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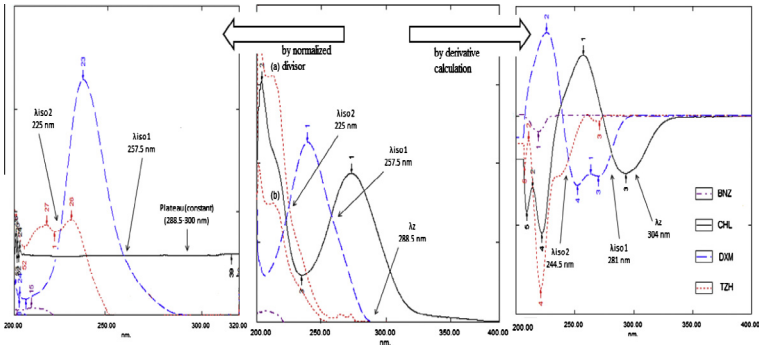
HIGHLIGHTS

- Three novel spectrophotometric methods are based on analysis of isosbestic point.
- Components at isosbestic point are determined via unified regression equation.
- They can be used for simultaneous analysis of complex ternary and quaternary mixtures.
- They do not need a special program and could be easily applied in QC labs.
- They are having equal accuracy, precision compared to PLS model.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 November 2013
Received in revised form 17 January 2014
Accepted 26 January 2014
Available online 12 February 2014

Keywords:
Isosbestic point
Absorption subtraction method
Amplitude modulation method
Amplitude summation method
Chloramphenicol
Dexamethasone

ABSTRACT

This work represents the application of the isosbestic points present in different absorption spectra. Three novel spectrophotometric methods were developed, the first method is the absorption subtraction method (AS) utilizing the isosbestic point in zero-order absorption spectra; the second method is the amplitude modulation method (AM) utilizing the isosbestic point in ratio spectra; and third method is the amplitude summation method (A-Sum) utilizing the isosbestic point in derivative spectra. The three methods were applied for the analysis of the ternary mixture of chloramphenicol (CHL), dexamethasone sodium phosphate (DXM) and tetryzoline hydrochloride (TZH) in eye drops in the presence of benzalkonium chloride as a preservative. The components at the isosbestic point were determined using the corresponding unified regression equation at this point with no need for a complementary method. The obtained results were statistically compared to each other and to that of the developed PLS model. The specificity of the developed methods was investigated by analyzing laboratory prepared mixtures and the combined dosage form. The methods were validated as per ICH guidelines where accuracy, repeatability, inter-day precision and robustness were found to be within the acceptable limits. The results obtained from the proposed methods were statistically compared with official ones where no significant difference was observed.

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http://dx.doi.org/10.1016/j.saa.2014.01.130
1386-1425/

Introduction

The term "isosbestic point" is usually employed to the intersection point of a set of absorption spectra, prepared in different ratios, in which the sum of the concentrations of two absorbing components, *X* and *Y*, is constant. The isosbestic point in zero order absorption spectra is known as isoabsorpative point, where the two absorbing components, *X* and *Y*, have equal absorptivity constants [1,2]. The literature review revealed the use of isosbestic point for the estimation of the fraction of a precursor in a complex formation or a conversion reaction [3–5]. The isoabsorptive point was also used for the determination of the total concentration of components in pharmaceutical binary mixtures with the aid of a complementary spectrophotometric method to determine one component concentration separately [6–8]. An isoabsorptive point will be retained in the ratio spectrum of the same components [9], where it has been combined with the ratio difference spectrophotometric method [10] for the simultaneous determination of a pharmaceutical binary mixture. Isosbestic point in ratio spectra was also utilized for the resolution of a mixture after derivitization [11]. The use of isosbestic point of derivative spectra was applied in the study of dissolution rate of aspirin to correct the interference that may originate from excipients [12].

The aim of the work is to develop three novel spectrophotometric methods based on the analysis of isosbestic point in different absorption spectra, either in zero order, ratio or derivative spectra. By applying simple mathematical manipulation, the isosbestic point analysis was done in order to identify the response corresponding to each component and then each component could be determined separately using the same regression equation (unified regression equation) with no need for a complementary spectrophotometric method. The first method was the absorbance subtraction method (AS), utilizing the isosbestic point in zero order absorption spectra; the second method was the amplitude modulation method (AM) utilizing the isosbestic point in ratio absorption spectra, and the third method was the amplitude summation method (A-Sum), utilizing the isosbestic point in derivative absorption spectra.

The drugs of interest were chloramphenicol (CHL), dexamethasone sodium phosphate (DXM) and tetryzoline hydrochloride (TZH). Several analytical techniques, including chromatography, spectrophotometry and electrochemistry, have been reported for the analysis of each of CHL [13–16], DXM [17–19] and TZH [20,21]. The binary mixture of CHL and DXM was determined using HPLC method [22]. No methods have been reported for the analysis of their ternary mixture. The structural formulae of the three components are shown in Fig. 1.

The three methods were applied to the pharmaceutical ternary mixture of the three drugs of interest. The determination of each component concentration was done with no interference of added excipients. PLS model was developed for the determination of the ternary mixture. The obtained results from the spectrophotometric methods were compared to each other and to that of PLS model to ensure their accuracy and precision.

Theory

Response factor

This factors is based on calculating a relationship between the response (either absorbance/or amplitude) of a component at two selected wavelengths [23,24]. For a mixture of two drugs *X* and *Y* having overlapped derivative spectra, where *Y* and *X* interfere at λ_1 ($V_{\lambda 1}$) and *X* does not show any contribution at another wavelength λ_2 ($V_{\lambda 2}$), the corresponding response factor of different concentration of pure *Y* is calculated at two selected wavelengths which represents the ratio between response value λ_1 (*V*1) and that at λ_2 (*V*2). It is constant for pure *Y*.

$$\text{Response Factor} = \left[\frac{V1}{V2}\right]$$

This factor could be used to calculate the response value of *Y* at interfering wavelength λ_1, after multiplication of the response factor by the response of the laboratory prepared mixture at λ_2 which show zero contribution of *X* as summarized in this equation:

$$\therefore \text{Response value of } Y \text{ in the mixture at } \lambda_1 = \left[\frac{V1}{V2}\right] \times V_{\lambda 2}(X+Y).$$

where $\left[\frac{V1}{V2}\right]$ is the response factor of pure *Y*; $V_{\lambda 2}(X+Y)$ is the response value of the mixture at λ_2.

Absorbance subtraction method (AS)

This method is based on the same principles as the absorption factor method [23]. However, this method could be applied for the analysis of a mixture of two drugs *X* and *Y* having overlapped spectra intersecting at isoabsorptive point where *Y* is extended over *X*, and *X* does not show any contribution at another wavelength (λ_2). In this method, the isoabsorptive point λ_{iso} could be used for separate quantitative estimation of each *X* and *Y* in their mixture (*X* + *Y*). The determination can be done using mathematically calculated factor of one of these components. By simple manipulation step, we can obtain the absorbance value corresponding to *X* and *Y*, separately. So, the concentration of each component could be obtained via the isoabsorptive point regression equation without any need for a complementary method.

The absorbance values corresponding to *X* and *Y* at λ_{iso} were calculated by using absorbance factor which is a constant for pure *Y* representing the average of ratio between the absorbance values of different concentrations of pure *Y* at λ_{iso} (abs $_{\lambda iso}$) to those at λ_2 (abs $_{\lambda 2}$).

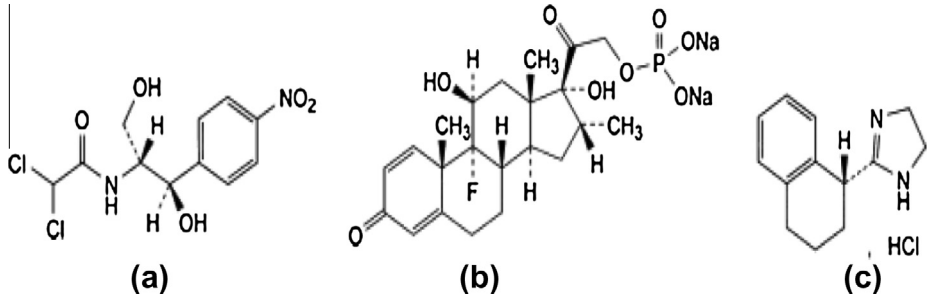

Fig. 1. The structural formulae of (a) chloramphenicol (CHL), (b) dexamethasone sodium phosphate (DXM) and (c) tetryzoline hydrochloride (TZH).