# A comparative study of progressive versus successive spectrophotometric resolution techniques applied for pharmaceutical ternary mixtures

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HIGHLIGHTS

- Novel progressive resolution technique: amplitude center method (ACM).
- Can be applied for severely overlapped and complex ternary and quaternary mixtures.
- The three components can be determined using only one single divisor.
- ACM does not need a special program and could be easily applied in QC labs.
- It can be applied for analysis of the dosage form with no inference of excipients.

GRAPHICAL ABSTRACT
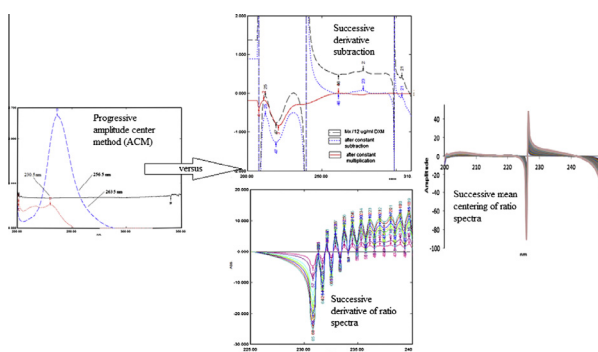
ARTICLE INFO

ABSTRACT

This work represents a comparative study of a novel progressive spectrophotometric resolution technique namely, amplitude center method (ACM), versus the well-established successive spectrophotometric resolution techniques namely; successive derivative subtraction (SDS); successive derivative of ratio spectra (SDR) and mean centering of ratio spectra (MCR). All the proposed spectrophotometric techniques consist of several consecutive steps utilizing ratio and/or derivative spectra. The novel amplitude center method (ACM) can be used for the determination of ternary mixtures using single divisor where the concentrations of the components are determined through progressive manipulation performed on the same ratio spectrum. Those 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 proposed methods were checked using laboratory-prepared mixtures and were successfully applied for the analysis of pharmaceutical formulation containing the cited drugs. The proposed methods were validated according to the ICH guidelines. A comparative study was conducted between those methods regarding simplicity, limitation and sensitivity. The obtained results were statistically compared with those obtained from the official BP methods, showing no significant difference with respect to accuracy and precision.

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Introduction

Analytical studies, related to the quality control and routine analysis of commercial products in the research or industry

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laboratories, use spectrophotometric methods such as derivative spectrophotometry [1,2], ratio spectra spectrophotometric [3–5] and other chemometric spectral calibration techniques [6,7]. These spectrophotometric methods are found to be preferable instead of hyphenated analytical instrumentations or techniques such as LC–MS, GC–MS, and LC–NMR which always require a prior step such as extraction and other tedious analytical process during analysis. On the other hand, the related techniques having complex components bring high cost and time consumption. Taking into account all those arguments, the quantitative spectrophotometric resolution of the mixtures of two or more compounds having overlapped spectra is an interesting and challenging issue for analytical chemists. For resolving the complex mixtures, the analytical chemist needs new analytical approaches to obtain accurate, precise and safe results.

The aim of this work was to develop a novel simple and accurate progressive spectrophotometric technique namely, amplitude center method (ACM), for the analysis of ternary mixtures. This novel technique was compared to well-established successive resolution techniques. All these techniques consist of consecutive steps applied in ratio and/or derivative spectra. The drugs of interest are 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 [8–11], DXM [12–14] and TZH [15,16]. The binary mixture of CHL and DXM was determined using HPLC method [17]. Lotfy et al. presented three spectrophotometric methods, based on isosbestic points, for the analysis of this ternary mixture [18]. The structural formulae of the three components of interest are shown in Fig. 1.

The novel progressive amplitude center method (ACM), together with the successive derivative subtraction (SDS); successive derivative of ratio spectra (SDR) and mean centering of ratio spectra (MCR) methods, were applied to the pharmaceutical ternary mixture of the three dugs of interest. The determination of each component concentration was done with no interference of added excipients. A comparative study is conducted between the developed methods to show their advantages and limitations.

Theory

Amplitude center method (ACM)

The amplitude center method depends on the progressive determination of three components in their ternary mixture through the same ratio spectrum via their corresponding amplitudes using single divisor. It consists of three stages complementary to each other namely; amplitude calculation, amplitude factor calculation and finally amplitude subtraction. Each of the mentioned methods was separately presented in previously reported spectrophotometric methods [4,19,20] which were applied for the analysis of binary mixtures only. Those three

methods were combined together for the analysis of ternary mixtures for the first time under the name of amplitude center method.

This novel method can be applied for a ternary mixture of X, Y and Z with partially or severely overlapped spectra, i.e. at λ_1 and λ_2, Y and Z spectra shows certain overlapping with no contribution of X, while all spectra are overlapped spectra at λ_3.

The first step is the amplitude calculation via amplitude difference method. For a mixture of three drugs $(X + Y + Z)$, Z can be determined by dividing the spectrum of the mixture by a known concentration of Z as a divisor (Z'). The division will give a new curve that can be summarized as follows:

$$\frac{X + Y + Z}{Z'} = \frac{X}{Z'} + \frac{Y}{Z'} + \frac{Z}{Z'} = \frac{X}{Z'} + \frac{Y}{Z'} + \text{constant}$$

where $\frac{Z}{Z'}$ is a constant which can be calculated according to the extent of the spectral overlapping of Y and Z by two different ways as follows:

1. Partial overlapping: If Z component is extended over Y and X is less extended than Y, so the amplitude $\frac{Z}{Z'}$ constant can be accurately determined at the extended region parallel to the wavelength axis, then the concentration of Z can be calculated using the regression equation representing correlation between the amplitudes of ratio spectra of the constant $\frac{Z}{Z'}$ at λ_1 and the corresponding concentration of Z. The amplitude related to component Y only at λ_2 can be calculated by subtracting the value of $\frac{Z}{Z'}$ constant from the amplitude of the binary mixture $(Y + Z)$ at the same wavelength where X shows no contribution, then the concentration of Y can be calculated using the regression equation representing correlation between the amplitudes of ratio spectra $\frac{Y}{Z'}$ at λ_2 and the corresponding concentration of Y.

2. Severe overlapping: If Z spectrum is severely overlapped with that of Y and both spectra are extended over X; or in case of mixtures containing low concentration of Z which will hinder the accurate determination of the value of $\frac{Z}{Z'}$ constant, then this constant amplitude and that corresponding to component Y can be calculated by amplitude difference method [20,21] using two selected wavelengths λ_1 and λ_2 on the obtained ratio curve of the mixture at the overlapping region of Y and Z, where X has no contribution. The difference between the ratio amplitudes at these two points $\left(\frac{Y}{Z'}\right)1$ and $\left(\frac{Y}{Z'}\right)2$ are calculated where the constant $\frac{Z}{Z'}$ will be cancelled along with any other instrumental error or any interference from the interfering substances X or Z. This amplitude difference will represent component Y only. This can be summarized as follows:

$$\Delta P = P_1 - P_2 = \left(\frac{Y}{Z'}\right)1 - \left(\frac{Y}{Z'}\right)2$$

where P_1 and P_2 are the ratio amplitudes of the ratio spectrum at λ_1 and λ_2.

The amplitude difference starts with computation of a regression equation representing the linear relationship between the difference of ratio amplitudes (ΔP) of different concentration of pure

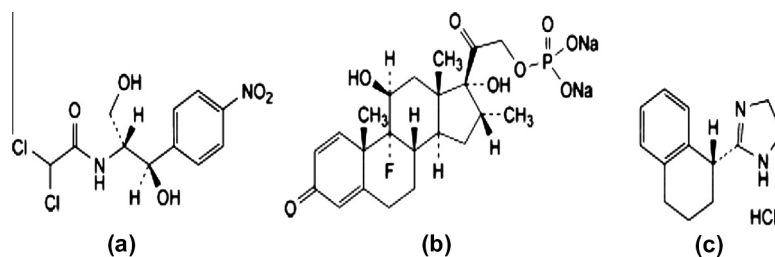**Fig. 1.** The structural formulae of (a) chloramphenicol (CHL), (b) dexamethasone sodium phosphate (DXM) and (c) tetryzoline hydrochloride (TZH).