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Novel two wavelength spectrophotometric methods for simultaneous determination of binary mixtures with severely overlapping spectra

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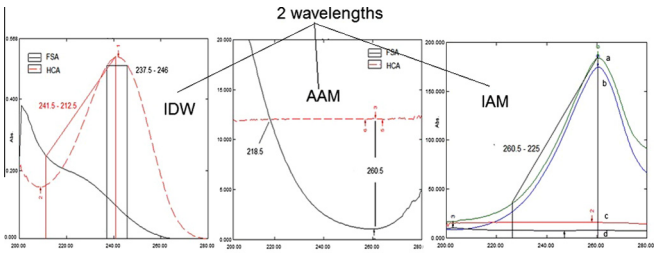
HIGHLIGHTS

- Four novel spectrophotometric methods using two wavelengths.
- They can be used for analysis of binary mixtures with severely overlapped spectra.
- They do not need a special program and could be easily applied in QC labs.
- They have equal accuracy, precision compared to chromatographic methods.
- They were applied for analysis of the dosage form without inference of excipients.

GRAPHICAL ABSTRACT



Bivariate method

λ1 \ λ2	212.5	218.5	228	235	241.5	250	260.5
212.5	0	0.9222	3.29	5.4666	6.7053	5.9885	2.455
218.5		0	2.3142	4.4892	5.8116	5.3592	2.2272
228			0	2.2974	3.9067	4.0913	1.785
235				0	1.809	2.6166	1.2516
241.5					0	1.2278	0.7228
250						0	0.196
260.5							0

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ABSTRACT

This work presents the application of different spectrophotometric techniques based on two wavelengths for the determination of severely overlapped spectral components in a binary mixture without prior separation. Four novel spectrophotometric methods were developed namely: induced dual wavelength method (IDW), dual wavelength resolution technique (DWRT), advanced amplitude modulation method (AAM) and induced amplitude modulation method (IAM). The results of the novel methods were compared to that of three well-established methods which were: dual wavelength method (DW), Vierordt's method (VD) and bivariate method (BV). The developed methods were applied for the analysis of the binary mixture of hydrocortisone acetate (HCA) and fusidic acid (FSA) formulated as topical cream accompanied by the determination of methyl paraben and propyl paraben present as preservatives. The specificity of the novel 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. No difference was observed between the obtained results when compared to the reported HPLC method, which proved that the developed methods could be alternative to HPLC techniques in quality control laboratories.

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Introduction

Quality control and routine analysis of commercial products in the research or industry laboratories tend to use spectrophotometric methods instead of hyphenated analytical instrumentations or techniques such as LC–MS, GC–MS and LC–NMR; as spectrophotometric methods do not require prior steps such as extraction and other tedious analytical process. For resolving the complex mixtures, the analytical chemist needs new spectrophotometric approaches to obtain accurate and precise results. Therefore, the analytical chemists have focused mainly on the use of new mathematical techniques or the use of the combined new approaches together with traditional analytical techniques.

Hydrocortisone acetate (HCA) is a corticosteroid with both glucocorticoid and to a lesser extent mineralocorticoid activity. It is used for topical application in the treatment of various skin disorders. A survey of the literature revealed the reported methods for the determination of HCA such as UV spectrophotometry [1,2], HPLC [3–5], TLC [6,7], micellar electrokinetic capillary chromatography [8,9] and capillary electrophoresis [10]. Fusidic acid (FSA) is an antimicrobial substance produced by the growth of certain strains of *Fusidium coccineum*. It is used topically in treatment of eye and skin infections. Different methods were reported for FSA such as UV spectrophotometry [11,12], HPLC [13,14] and TLC [15]. Several methods were reported for the determination of the preservatives (MPB and PPB) in different pharmaceutical formulations [3,16–18]. All those components are official [19]. Two HPLC methods were reported for the determination of HCA and FSA [13,20], but no spectrophotometric methods has been developed for the analysis of this mixture. The structural formulae of the components of interest are shown in Fig. 1.

Different spectrophotometric methods were developed based on two wavelength technique through the use of the absorbance or amplitude at two wavelengths and record the difference of the values at these wavelengths to permit either resolution of the overlapped spectra in order to help in the analysis of complex mixtures, or measurement of the individual components in the mixtures with satisfactory degree of accuracy and precision. Several methods could be applied such as: dual wavelength [21,22], absorption correction [23], absorbance ratio [2,24], bivariate calibration [25,26], ratio difference [27,28], constant center [29,30], Vierordt's [31,32], absorbance subtraction and amplitude modulation [33–35].

The aim of the work is to develop four novel spectrophotometric methods based on the measurements done at two wavelengths, either in zero order or ratio spectra, for the determination of binary mixture of severely overlapped spectral components which are: hydrocortisone acetate (HCA) and fusidic acid (FSA) formulated as topical cream in presence of methyl paraben (MPB) and propyl paraben (PPB) as preservatives. The developed novel methods are: induced dual wavelength method (IDW), dual wavelength resolution technique (DWRT), advanced amplitude modulation method (AAM) and induced amplitude modulation method (IAM). The results of the novel methods are compared to that of three well-established methods which are: dual wavelength method (DW), bivariate method (BV) and Vierordt's method (VD).

The proposed spectrophotometric methods were successfully applied to the pharmaceutical binary mixture of HCA and FSA after the spectral resolution of the preservatives (MPB and PPB), followed by the simultaneous determination of those preservatives. The obtained results from the developed methods were compared to each other and to the reported HPLC method [13] to ensure their accuracy and precision.

Theory

Induced dual wavelength method (IDW)

This method can be applied for a binary mixture of *X* and *Y* with complete overlapped zero order absorption spectra at two wavelengths λ_1 and λ_2, where the absorbance of the interfering substance between those two wavelengths are not equal (absorbance difference does not equal zero), so the conventional dual wavelength method cannot be applied. This can be illustrated by the following equations:

$$A_1 = A_{X1} + A_{Y1} \quad \text{at } \lambda_1 \tag{1}$$

$$A_2 = A_{X2} + A_{Y2} \quad \text{at } \lambda_2 \tag{2}$$

where A_1 represents the absorbance of the mixture at λ_1 (λ_{max} of *X*), while A_2 is the absorbance at any other wavelength (λ_2). To cancel the effect of *Y* at the two selected wavelengths, the equality factor of pure *Y* at these wavelengths (F_Y) is calculated:

$$F_Y = A_{Y1}/A_{Y2} \quad \therefore A_{Y1} = F_Y A_{Y2}$$

By substituting in Eq. (1)

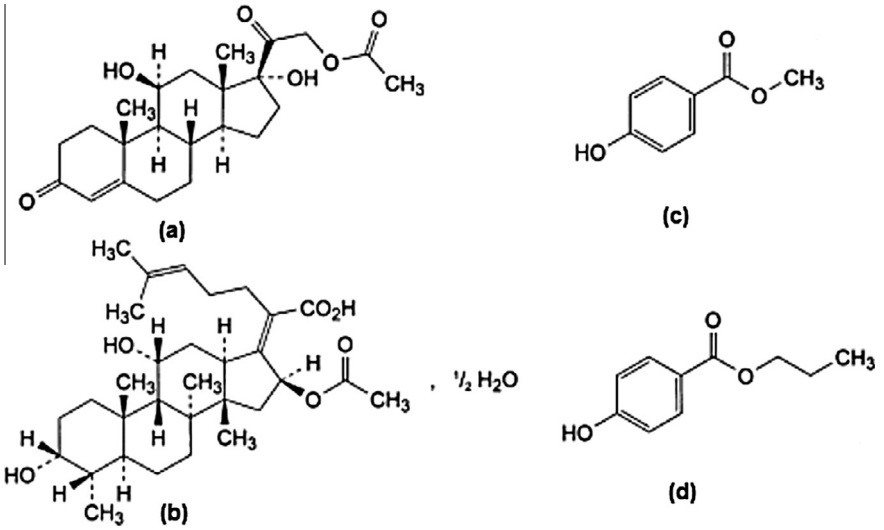

Fig. 1. The structural formulae of (a) Hydrocortisone acetate (HCA), (b) fusidic acid (FSA), (c) methyl paraben (MP) and (d) propyl paraben (PP).