A comparative study of ICH validated novel spectrophotometric techniques for resolving completely overlapping spectra of quaternary mixtures

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ARTICLE INFO

ABSTRACT

A pharmaceutically marketed mixture of Yohimbine, Alpha-tocopheryl acetate, Niacin, and Caffeine co-formulated as a promising therapy for erectile dysfunction. Simultaneous determination of the aforementioned pharmaceutical formulation without prior separation steps was applied using mean centering of ratio spectra and triple divisor spectrophotometric methods. Mean centering of ratio spectra method depended on using the mean centered ratio spectra in three successive steps which eliminated the derivative steps and so the signal to noise ratio was improved. The absorption spectra of the prepared solutions were measured in the wavelength range of 215–300 nm in the concentration ranges of 1–15, 3–15, 1–20, and 3–15 $\mu g\ mL^{-1}$ for Yohimbine, Alpha-tocopheryl acetate, Niacin, and Caffeine, respectively. The amplitudes of the mean centered third ratio spectra were measured at 250 nm and 268 nm for Yohimbine and Alpha-tocopheryl acetate, respectively and at peak to peak 272–273 and 262–263 nm for Niacin and Caffeine, respectively. In triple divisor method each drug in the quaternary mixture was determined by dividing the spectrum of the quaternary mixture by a standard spectrum of a mixture containing equal concentrations of the other three drugs. First derivative of these ratio spectra was obtained where determination could be achieved without any interference from the other three drugs. Amplitudes of 1–15, 3–15, 1–15, and 3–15 $\mu g\ mL^{-1}$ were used for selective determination of Yohimbine, Alpha-tocopheryl acetate, Niacin, and Caffeine, respectively.

Laboratory prepared mixtures were analyzed by the developed novel methods to investigate their selectivity also, Super Act® capsules were successfully analyzed to ensure absence of interference from additives. The developed methods were validated according to the ICH guidelines. The proposed methods were statistically compared with each other and with the reported methods; using student t-test, F-test, and one way ANOVA, where no significant difference was found with respect to accuracy and precision.

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1. Introduction

Yohimbine (YH) is chemically known as 17α-Hydroxy-20-α-yohimban-16-ß-carboxylic acid, methyl ester, hydrochloride, [1]. It is a bark extract of *Corynanthe yohimbe* tree. It is a mild monoamine oxidase inhibitor (MAOI) so it has aphrodisiac effect; it also has hypotensive, cardiovascular depressant, and hypnotic effects [2]. Alpha-tocopheryl acetate, Vitamin E acetate, (VE) is chemically known as 5,7,8-trimethyltocol acetate [1,3]. It is an extract from soybean oil used for Vitamin E deficiency [2]. It is a potent antioxidant useful in prevention of atherosclerosis, neuropathy, retinopathy, and cerebro- and peripheral vascular diseases [4]. Vitamin B₃ (VB) also known as nicotinic acid or Niacin is chemically known as 3-Pyridinecarboxylic acid [1,3]. It is found in many foods and used for high cholesterol treatment. It is also used along with other treatments for circulation problems, headache, migraine, dizziness, and to reduce the diarrhea associated with cholera

[2]. Caffeine (CAF) is chemically known as, 1,3,7-trimethylxanthine [1, 3], that inhibits phosphodiesterase enzyme; it has an antagonistic effect at central adenosine receptors so acts as a central nervous system stimulant. Also, it has wide uses in analgesic preparations to enhance the effects of both opioid and non-opioid analgesics [2]..

Combination of YH, VE, VB, and CAF medications is formulated as Super Act® capsules which work to treat male sexual dysfunction related to vascular or diabetic origins. YH is primarily used as a treatment for erectile dysfunction [1]. Addition of VE enhances YH sexual effect through improving circulation and repairing tissue damage [5] while VB improves the erectile function [6]. Also CAF was associated with a higher potency rate in men [7]. The only interaction in the studied formulation, Super Act® capsule, is that both YH and CAF decrease sedation [8].

In USP [1], YH, VE, VB, and CAF were assayed by HPLC, gas chromatography (GC), spectrophotometry, and HPLC, respectively while in B. P. [3], VE was assayed by GC while VB and CAF were assayed by titration.

The literature survey reveals some analytical methods for determination of YH either alone or in combination with other drugs such as

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stability indicating UV-spectrophotometric [9], Spectrofluorimetric [10], chromatographic [9,11–14], and electrochemical methods [15]. Other methods were reported for determination of VE such as, spectroscopic [16,17] and chromatographic [18–20] methods while VB was determined by spectroscopic [21] and chromatographic [21–27] methods. The literature survey showed so many methods for determination of CAF either alone or with other drugs but no methods were reported for its determination with YH, VE, or VB. After meticulous research, no analytical methods were reported in literature for determination of the quaternary mixture under study.

As the number of drugs in pharmaceutical formulation increases, their spectral resolution without preliminary separation becomes more difficult. This manuscript aimed to develop two new validated spectrophotometric methods namely mean centering of ratio spectra (MCR) and triple divisor (TD) spectrophotometric methods for resolving quaternary mixtures with completely overlapped spectra. The developed MCR method is an extension of the commonly used mean centering of ratio spectra spectrophotometric method commonly used for resolving binary and ternary mixtures [28–38] where it is used to remove the contribution of co-formulated components from data matrix precisely.

Triple divisor (TD) spectrophotometric technique is an extension and a modification of the commonly applied double divisor spectrophotometric method [39–41]. This modification enabled determination of any component in a quaternary mixture in two steps only; firstly, division of the quaternary mixture spectrum by the spectrum of a mixture of the other three components. Secondly, derivatization of the obtained ratio spectrum where this component can be measured at any wavelength of maximum or minimum absorbance. Validity of the proposed new methods was ascertained by applying them for determination of a quaternary mixture containing YH, VE, VB, and CAF.

The developed spectrophotometric methods have advantages over chromatographic methods as that they do not need sophisticated apparatus or high coast solvents. They are time-saving and do not need sample extraction steps or long chromatographic retention time. Therefore, spectrophotometry can be used as an alternative method for resolving quaternary mixtures with completely overlapping spectra in pharmaceutical quality control laboratories. The two novel spectrophotometric methods were validated according to ICH guidelines [42].

1.1. Theoretical background

1.1.1. Mean centering of ratio spectra spectrophotometric method

Issa et al. [35] explained the theoretical background of MCR method used for resolving quaternary mixtures as follows: if a mixture of four compounds, YH, VE, VB, and CAF, are considered where Beer's law is obeyed over the whole wavelength range used for all compounds, then

$$A_m = \propto_{YH} C_{YH} + \propto_{VE} C_{VE} + \propto_{VB} C_{VB} + \propto_{CAF} C_{CAF} \qquad (1)$$

where A_m is the mixture absorbance vector, $\propto_{YH}$, $\propto_{VE}$, $\propto_{VB}$ and $\propto_{CAF}$ are the absorptivity vectors, C_{YH}, C_{VE}, C_{VB} and C_{CAF} are the concentrations of YH, VE, VB, and CAF in the mixture, respectively.

For determination of YH, if Eq. (1) is divided by $\propto_{VE}$ corresponding to the spectrum of a standard solution of VE, the first divisor, so the first ratio spectrum is obtained

$$\frac{A_m}{\propto_{VE}} = \frac{\propto_{YH} C_{YH}}{\propto_{VE}} + C_{VE} + \frac{\propto_{VB} C_{VB}}{\propto_{VE}} + \frac{\propto_{CAF} C_{CAF}}{\propto_{VE}}. \qquad (2)$$

If the Eq. (2) is mean centered, then

$$mc\,\frac{A_m}{\propto_{VE}} = mc\,\frac{\propto_{YH} C_{YH}}{\propto_{VE}} + mc\,\frac{\propto_{VB} C_{VB}}{\propto_{VE}} + mc\,\frac{\propto_{CAF} C_{CAF}}{\propto_{VE}}. \qquad (3)$$

If Eq. (3) is divided by $mc\,\frac{\propto VB}{\propto VE}$, the second divisor, the second ratio spectrum is obtained

$$\frac{mc\,\frac{A_m}{\propto VE}}{mc\,\frac{\propto VB}{\propto VE}} = \frac{mc\,\frac{\propto YH\ C_{YH}}{\propto VE}}{mc\,\frac{\propto VB}{\propto VE}} + C_{VB} + \frac{mc\,\frac{\propto CAF\ C_{CAF}}{\propto VE}}{mc\,\frac{\propto VB}{\propto VE}}. \qquad (4)$$

If Eq. (4) is mean centered, then

$$mc\,\frac{mc\,\frac{A_m}{\propto VE}}{mc\,\frac{\propto VB}{\propto VE}} = mc\,\frac{mc\,\frac{\propto YH\ C_{YH}}{\propto VE}}{mc\,\frac{\propto VB}{\propto VE}} + mc\,\frac{mc\,\frac{\propto CAF\ C_{CAF}}{\propto VE}}{mc\,\frac{\propto VB}{\propto VE}}. \qquad (5)$$

And if Eq. (5) is divided by $mc(\frac{mc\frac{\propto CAF}{\propto VE}}{mc\frac{\propto VB}{\propto VE}})$, the third divisor, this will results in the third ratio spectrum,

$$mc\,\frac{mc\,\frac{A_m}{\propto VE}}{mc\,\frac{\propto VB}{\propto VE}} / mc\,\frac{mc\,\frac{\propto CAF}{\propto VE}}{mc\,\frac{\propto VB}{\propto VE}} = mc\,\frac{mc\,\frac{\propto YH\ C_{YH}}{\propto VE}}{mc\,\frac{\propto VB}{\propto VE}} / mc\,\frac{mc\,\frac{\propto CAF}{\propto VE}}{mc\,\frac{\propto VB}{\propto VE}} + C_{CAF}. \qquad (6)$$

If Eq. (6) is mean centered, then

$$X = Z\ C_{YH}, \qquad (7)$$

where, $X = mc\,(mc\,\frac{mc\frac{A_m}{\propto VE}}{mc\frac{\propto VB}{\propto VE}} / \frac{mc\frac{\propto CAF}{\propto VE}}{mc\frac{\propto VB}{\propto VE}})$ and $Z = mc\,(mc\,\frac{mc\frac{\propto YH}{\propto VE}}{mc\frac{\propto VB}{\propto VE}} / \frac{mc\frac{\propto CAF}{\propto VE}}{mc\frac{\propto VB}{\propto VE}})$.

Practically, the signal of mean centered third ratio spectrum obtained in Eq. (7) is dependent only on the concentration value C_{YH}, but is independent on the concentration values of C_{VE}, C_{VB}, and C_{CAF} in the mixture. The concentrations of the other components (VE, VB, and CAF) can be determined separately by analogous procedures to that of YH.

1.1.2. Triple divisor spectrophotometric method

In this method, for determination of YH in a multicomponent mixture containing YH, VE, VB, and CAF, the stored spectra of the mixture were divided by the sum standard spectra of equal concentrations of VE, VB, and CAF as 'a triple divisor' then, the first derivative of the resulted ratio spectra was obtained.

$$As, A_m = \propto_{YH} C_{YH} + \propto_{VE} C_{VE} + \propto_{VB} C_{VB} + \propto_{CAF} C_{CAF}. \qquad (1)$$

And C^0_{VE}, C^0_{VB}, and C^0_{CAF} are of equal concentration (C^0 is standard concentration) so,

$$\frac{d}{d\lambda}\frac{A_m}{\propto_{VE} C^0_{VE} + \propto_{VB} C^0_{VB} + \propto_{CAF} C^0_{CAF}} = \frac{d}{d\lambda}\frac{\propto_{YH} C_{YH} + \propto_{VE} C_{VE} + \propto_{VB} C_{VB} + \propto_{CAF} C_{CAF}}{\propto_{VE} C^0_{VE} + \propto_{VB} C^0_{VB} + \propto_{CAF} C^0_{VE}} \qquad (8)$$

$$\frac{d}{d\lambda}\frac{A_m}{\propto_{VE} C^0_{VE} + \propto_{VB} C^0_{VB} + \propto_{CAF} C^0_{CAF}}$$

$$= \frac{d}{d\lambda}\left[\frac{\propto_{YH} C_{YH}}{\propto_{VE} C^0_{VE} + \propto_{VB} C^0_{VB} + \propto_{CAF} C^0_{VE}} + Constant\right]$$

$$= \frac{d}{d\lambda}\left[\frac{\propto_{YH} C_{YH}}{\propto_{VE} C^0_{VE} + \propto_{VB} C^0_{VB} + \propto_{CAF} C^0_{VE}}\right] + Zero.$$

From the last equation, the amplitudes measured are dependent only on concentration values, C_{YH}, C^0_{VE}, C^0_{VB}, and C^0_{CAF} (C^0 is standard concentration), but are independent on the concentration values C_{VE}, C_{VB}, and C_{CAF} in the quaternary mixture.