Comparative study of spectrophotometric methods manipulating ratio spectra: An application on pharmaceutical binary mixture of cinnarizine and dimenhydrinate

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**Highlights**
- Four spectrophotometric methods for determination of cinnarizine and dimenhydrinate.
- They can be used for analysis of binary mixtures with severely overlapped spectra.
- They have equal accuracy, precision compared to chromatographic methods.
- Ratio difference does not need fixed wavelengths or any derivative calculation.
- Constant center method predicts the UV spectra of each single drug.

**Graphical Abstract**

**Abstract**
Four simple, specific, accurate and precise spectrophotometric methods are developed and validated for simultaneous determination of cinnarizine (CIN) and dimenhydrinate (DIM) in a binary mixture with overlapping spectra, without preliminary separation. The first method is dual wavelength spectrophotometry (DW), the second is a ratio difference spectrophotometric one (RD) which measures the difference in amplitudes between 250 and 270 nm of ratio spectrum, the third one is novel constant center spectrophotometric method (CC) and the fourth method is mean centering of ratio spectra (MCR). The calibration curve is linear over the concentration range of 4–20 and 10–45 μg/ml for CIN and DIM, respectively. These methods are tested by analyzing synthetic mixtures of the above drugs and they are applied to commercial pharmaceutical preparation of the subjected drugs. The validity of results was assessed by applying standard addition technique. The results obtained were found to agree statistically with those obtained by a reported method, showing no significant difference with respect to accuracy and precision.

**Introduction**
Cinnarizine is 1-(diphenylmethyl)-4-(3-phenyl-2-propenyl)piperazine (CIN) [1], (Fig. 1a). It is a piperazine derivative with antihistamine, sedative, and calcium-channel blocking activity. It is used for the symptomatic treatment of nausea and vertigo caused by Ménière’s disease and other vestibular disorders and for the prevention and treatment of motion sickness [2]. CIN is official in British Pharmacopeia and determined by potentiometric titration and liquid chromatographic method [3].

Literature survey reveals few methods that have been reported for the determination of CIN in pharmaceutical formulations,
biological samples and with other drugs in combination; including colorimetry [4], potentiometric titration [5], spectrophotometry [6], RP-HPLC [7] and HPTLC [8].

Dimenhydrinate is 2-benzhydryloxy-N,N dimethyl-ethanamine; 8-chloro-1,3-dimethyl-7H-purine-2,6-dione (DIM) (Fig. 1b) [1]. It is 8-chlorotheophylline salt of diphenhydramine and is used to prevent motion sickness [9]. It is official in British Pharmacopoeia and determined by potentiometric titration and liquid chromatographic method [3]. Few methods have been reported for the determination of DIM in pharmaceutical formulations, biological samples and with other drugs in combination including spectrophotometry [10] and HPLC [11–13].

Very few methods have been reported for analysis of binary mixture of CIN and DIM, including spectrophotometry [14–16] and HPLC [17]. The aim of this work was to develop a comparative study of smart, sensitive and validated spectrophotometric methods for the simultaneous determination of CIN and DIM either in bulk, laboratory prepared mixtures and pharmaceutical dosage form. The spectrophotometric methods applied are dual wavelength, two newly developed spectrophotometric methods; ratio difference and constant center and mean centering. The advantage of these methods is the simultaneous determination of both drugs without need for derivatization procedures, expensive solvents or large number of samples therefore can be successfully applied in quality control laboratories.

Theory of the proposed methods

Ratio difference spectrophotometric method (RD)

Recently, Hayam et al. [18,19] developed a new simple, rapid and selective method for the simultaneous determination of components having overlapping spectra in binary mixtures, having the advantages of minimal data processing and wider range of application over other conventional spectrophotometric methods.

Theory for the constant center

This novel method [20,21] depends on using smart original mathematical techniques utilizing the constants present in the ratio spectra which could be adapted to obtain the original spectra of both components in the binary mixture and analyze them at their $\lambda_{\text{max}}$ with maximum accuracy and reproducibility. This method considered as a new approach of the ratio difference method to enable constant value calculation. This new method is very simple, accurate, precise and do not require any sophisticated apparatus or computer programs.

Experimental

Instruments

Spectrophotometer: SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV–1650 PC connected to IBM compatible and a HP1020 laserjet printer. The bundled software, UV-Probe personal spectroscopy software version 2.21(SHIMADZU) is used. The spectral band is 2 nm and scanning speed is 2800 nm/min with 0.1 nm interval.

Software

Matlab® version 7, release 14.

Chemicals and reagents

Pure samples are kindly supplied by Amoun Pharmaceutical company, Cairo, Egypt. Their purity is found to be 100.24 ± 0.961 and 100.80 ± 1.245, for CIN and DIM, respectively, according to a reported spectrophotometric method [14].

Pharmaceutical formulations: Amocerebral plus tablets Batch No. 124820, are kindly supplied by Amoun Pharmaceutical company, Cairo, Egypt and are claimed to contain 20 mg of CIN and 40 mg of DIM per each tablet.

Methanol: Spectroscopy grade is purchased from El-NASR Pharmaceutical Chemicals Co., Abu-Zaabal, Cairo, Egypt.

Standard solutions

CIN and DIM standard solutions (both are 0.1 mg/ml), are prepared by dissolving 10 mg of CIN and DIM, respectively, in a few milliliters of methanol into a 100-ml volumetric flasks and then completing to volume with the same solvent.

Procedures

Spectral characteristics of CIN and DIM

The absorption spectra of the two compounds are recorded over the range 200–400 nm using methanol as a blank.

Linearity and construction of calibration curves

Aliquots equivalent to (40–200 µg) and (100–450 µg) of CIN and DIM, respectively are separately transferred from their standard solutions (0.1 mg/ml, each) into two series of 10-ml volumetric flasks. Then complete to volume with methanol. The spectra of the prepared standard solutions are scanned from 200 to 400 nm and stored in the computer.

For dual wavelength method (DW). Calibration curves are constructed between the difference in absorbance at 237 nm, 249 nm and 286 nm, 292 nm for CIN and DIM, respectively against their corresponding concentrations. The regression equations for both drugs are computed.

For ratio difference spectrophotometric method (RD). The stored spectra of CIN are divided by the spectrum of 10 µg/ml DIM while DIM spectra are divided by the spectrum of 12 µg/ml CIN.

Calibration curves of CIN and DIM are constructed by plotting the difference between the amplitudes of ratio spectra at 250 and 270 nm for CIN and DIM, versus the corresponding concentrations and the regression equations are computed.
For constant center method (CC). Construct two calibration curves relating the absorbance of the zero order spectra of CIN at 250 nm versus the corresponding concentrations of CIN and DIM at 270 nm versus the corresponding concentrations of DIM, the regression equations are computed.

The stored spectra of CIN are divided by the spectrum of 10 μg/ml DIM while DIM spectra are divided by the spectrum of 12 μg/ml CIN. The obtained ratio spectra are recorded. Construct Calibration curves by plotting the difference between the amplitudes of the obtained ratio spectra at [250 nm and 270 nm] versus amplitudes of ratio spectra at 250 nm, 270 nm for CIN and DIM; respectively and the regression equations are computed.

Mean centering of ratio spectra method (MCR). The scanned spectra are exported to Matlab for subsequent calculation, then the spectra of CIN are divided by the absorption spectrum of standard solution of DIM (10 μg/ml), the obtained ratio spectrum is then mean centered. Also the spectra of DIM are divided by the absorption
spectrum of standard solution of CIN (12 μg/ml) and the obtained ratio spectra are mean centered. The calibration curves for CIN and DIM are constructed, each by plotting the mean centered values at 249 nm and 280 nm, for CIN and DIM, respectively versus their corresponding concentrations and the regression equations are computed.

**Analysis of laboratory prepared mixtures**

For preparation of laboratory mixtures, into a series of 10-mL volumetric flasks, aliquots equivalent to 40–200 μg of CIN and 100–450 μg of DIM are accurately transferred from their standard solutions (each, 0.1 mg/ml) with different ratios of the two drugs and the volume is completed with methanol. The spectra of the prepared mixtures are scanned from 200 to 400 nm and stored in the computer.

For dual wavelength method, the differences in absorbance at 237 nm, 249 nm for CIN 286 nm, 292 nm for DIM are recorded. For ratio difference spectrophotometric method, the stored spectra of different laboratory prepared mixtures were divided separately by the absorption spectra of standard DIM (10 μg/ml) and standard CIN (12 μg/ml). The ratio spectra were recorded at 250 nm and 270 nm for CIN and DIM. The concentrations of the drugs were calculated from the computed regression equations.

For constant center method, the stored spectra of different laboratory prepared mixtures are divided separately by the absorption spectra of standard DIM (10 μg/ml) and standard CIN (12 μg/ml). The ratio spectra are recorded at 250 nm and 270 nm, the difference in the amplitudes between 250 nm and 270 nm is used to calculate the postulated value of the ratio amplitude at 250 nm, 270 nm for each mixture. The constant of each mixture is calculated by subtracting the postulated value from the recorded value of each mixture at 250 nm, 270 nm. Multiply the obtained constant values of CIN and DIM for each mixture by the spectra of 12 μg/ml standard CIN and 10 μg/ml standard DIM; respectively, so the original spectra of CIN and DIM are obtained. The concentration of each drug is calculated using its corresponding regression equation.

For mean centering method (MCR), the spectra of the prepared solutions of the two series are recorded from 200 to 400 nm and stored in the computer then process them via matlab as under the proposed method. The concentration of each drug was calculated using the specified regression equation.

**Application to pharmaceutical preparation**

To determine the content of CIN and DIM in commercial tablets (each tablet labeled to contain 20 mg CIN and 40 mg DIM), 20

![Fig. 4. Ratio spectra of DIM (10–45 μg/ml) using 12 μg/ml of CIN as a divisor.](image)

For constant center method, the stored spectra of different laboratory prepared mixtures are divided separately by the absorption spectra of standard DIM' (10 μg/ml) and standard CIN' (12 μg/ml). The ratio spectra are recorded at 250 nm and 270 nm, the difference in the amplitudes between 250 nm and 270 nm is used to calculate the postulated value of the ratio amplitude at 250 nm, 270 nm for each mixture. The constant of each mixture is calculated by subtracting the postulated value from the recorded value of each mixture at 250 nm, 270 nm. Multiply the obtained constant values of CIN and DIM for each mixture by the spectra of 12 μg/ml standard CIN' and 10 μg/ml standard DIM'; respectively, so the original spectra of CIN and DIM are obtained. The concentration of each drug is calculated using its corresponding regression equation.

![Fig. 5. Ratio spectra of CIN 20 μg/ml (---), 10 μg/ml of DIM (.....), and laboratory-prepared mixture of both (with the same concentration of each) (...) using 10 μg/ml of DIM as a divisor showing that the difference of peak amplitudes of ratio spectra at λ₁ and λ₂ is constant for pure CIN and the laboratory-prepared mixture containing same concentration.](image)
Tablets are weighed and finely powdered. A portion of powder equivalent to one tablet is weighed accurately and transferred to a 100-ml beaker. 50 ml of methanol is added, stirred using a magnetic stirrer for 30 min and filtered through 0.5 μm Whatman filter paper into a 100-ml volumetric flask. The residue is washed three times each with 10 ml of methanol and the solution is completed to the mark with the same solvent. From the above prepared solution, further dilutions are done using the same solvent to obtain a solution claimed to contain 20 μg/ml for CIN and 40 μg/ml for DIM.

The proposed methods are applied for the analysis of the studied drugs in their pharmaceutical formulation using the procedures mentioned under analysis of laboratory prepared mixtures for each method and the concentrations of the cited drugs are calculated from the corresponding regression equations.

Results and discussion

The main task of this work is to establish new, simple, sensitive and accurate analytical methods for simultaneous determination of CIN and DIM in their bulk powders and pharmaceutical dosage form with satisfactory accuracy and precision. As well, the ability of the proposed methods to determine both drugs in their pure form, laboratory prepared mixtures and in their pharmaceutical formulations.

By scanning the absorption spectra of CIN and DIM in methanol, severely overlapped spectral bands are observed in the wavelength region of 200–300 nm which hinders their direct determination (Fig. 2), so different methods are applied for achieving best resolution and quantitative determination of each drug without any interference from the other.

**Dual wavelength method (DW)**

The principle for DW method is that the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of component A; while for component B the absorbance difference equals to zero. For the determination of CIN two wavelengths 237 nm, 249 nm are selected where the absorbance difference between the two wavelengths is directly proportional to the concentration of CIN and the absorbance difference of DIM at these wavelengths is zero.

For the determination of DIM two wavelengths 286 nm, 292 nm are selected where the absorbance difference between the two wavelengths is directly proportional to the concentration

Fig. 6. Ratio spectra of DIM 30 μg/ml (-----), 12 μg/ml of CIN (-----), and laboratory-prepared mixture of both (with the same concentration of each)(---) using 12 μg/ml of CIN as a divisor showing that the difference of peak amplitudes of ratio spectra at λ1 and λ2 is constant for pure DIM and the laboratory-prepared mixture containing same concentration.

![Graph showing the linear correlation between amplitude difference (P270nm - P250nm) of DIM ratio spectra against the amplitude at 270 nm.](image)

\[ y = 0.9092x - 0.0078 \]
\[ r = 0.9999 \]
of DIM and the absorbance difference of CIN at these two wavelengths is zero (Fig. 2). Difference in absorbances of CIN at 237 and 249 nm are plotted against its concentration in the range of 4–20 \( \mu \text{g/ml} \), also for DIM, difference in absorbances at 286 and 292 nm are plotted against its concentration in the range of 10–45 \( \mu \text{g/ml} \).

The concentrations of CIN and DIM can be calculated from the following regression equations:

\[
A_{\text{CIN}} = 0.013C - 0.0014 \quad r = 0.9997
\]

\[
A_{\text{DIM}} = 0.01C + 0.0069 \quad r = 0.9998
\]

where \( A \) is the absorbance difference, \( C \) is concentration (\( \mu \text{g/ml} \)) and \( r \) is the correlation coefficient.

**Ratio difference spectrophotometric method (RD)**

The most striking feature of the ratio difference method is its simplicity, rapidity and accuracy [18]. This is a newly developed method having the ability for solving severely overlapped spectra without prior separation; meanwhile it doesn’t require any sophisticated apparatus or expensive computer programs.

The utilization of ratio difference method is to calculate the unknown concentration of a component of interest present in a mixture containing both the component and an interfering component. The ratio spectra of CIN using spectrum of 10 \( \mu \text{g/ml} \) DIM as a divisor is shown in Fig. 3, while DIM ratio spectra using the CIN spectrum of 12 \( \mu \text{g/ml} \) as a divisor is shown in Fig. 4.

The only requirement in the ratio difference method is the contribution of the two overlapped spectra at the two selected wavelengths \( \lambda_1 \) and \( \lambda_2 \) where the ratio spectrum of the interfering component shows the same amplitude (constant) whereas the component of interest shows significant difference in these two amplitude values at these two selected wavelengths with concentration. Similarly, the selected wavelengths are used for the estimation of the second component. Thus, the overlapped spectra of the cited drugs suggested that a ratio difference method was a suitable method for simultaneous determination of CIN and DIM. Ratio difference method starts by scanning the zero order absorption spectra of the laboratory-prepared mixtures (CIN and DIM). For determination of CIN, divide the previously scanned ratio spectra by a carefully chosen concentration of standard DIM’ (10 \( \mu \text{g/ml} \)) as a divisor to produce new ratio spectra which represent CIN/DIM’ + constant as shown in Fig. 5. The amplitudes at 250 nm and 270 nm were selected. The amplitudes at these two wavelengths were subtracted, so the constant DIM/DIM’ will be cancelled. The concentration of CIN was calculated using the corresponding regression equation (obtained by plotting the difference in the amplitudes at 250 nm and 270 nm of the ratio spectra of CIN/DIM’ against the corresponding concentrations). Similarly, the two
selected wavelengths for the estimation of DIM using standard CIN (12 µg/ml) as a divisor shown in Fig. 6.

The regression equations for amplitude difference (250–270 nm) were computed and found to be:

\[
RD_{\text{CIN}} = 0.4375C + 0.0575 \quad r = 0.9997 \\
RD_{\text{DIM}} = 0.1527C + 0.0931 \quad r = 0.9998
\]

where RD is the amplitude difference, C is concentration (µg/ml) and r is the correlation coefficient. The mean percentage recoveries were 100.04 ± 1.340 and 99.84 ± 0.990, for CIN and DIM, respectively.

**Constant center method (CC)**

In this work a simple and recently developed method; namely constant center spectrophotometric method is applied for resolving mixtures with spectral overlapping as CIN and DIM.

For the determination of CIN in the binary mixture, the ratio spectra of the mixtures obtained by the absorption spectra of 12 µg/ml CIN as a divisor (DIM/CIN) + constant. Ratio difference at two selected wavelength [250 nm (λ) and 270 nm (λ2)] is calculated [(DIM/CIN) – (DIM/CIN)2], where the interfering substance (CIN) is cancelled and subsequently shows no interference. The practical ratio amplitude of the mixtures at 250 nm are recorded [(DIM/CIN) + (CIN/CIN)2] for each laboratory prepared mixture, while the postulated ratio amplitude value of (DIM/CIN) can be calculated using the equation representing the linear relationship between the ratio difference of ratio spectra at 250 nm and 270 nm versus the corresponding ratio amplitudes at 270 nm as shown in (Fig. 7).

\[
P_1 - P_2 = 0.9092P_1 - 0.0078 \quad r = 0.9999
\]

where \(P_1\), \(P_2\) are the ratio amplitudes at 250 nm and 270 nm of the ratio spectra of different concentration of DIM (10–45 µg/ml) using 12 µg/ml CIN as a divisor.

The constant value is calculated by monitoring the effect on the amplitude of the ratio spectrum of DIM at 250 nm. \(AP = (P_{\text{recorded}} - P_{\text{postulated}})\), so the constant value is calculated by measuring the difference between the recorded amplitude and postulated amplitude at this wavelength.

\[
C.V = |P_{\text{recorded}} - P_{\text{postulated}}|
\]

where C.V is the constant value, \(P_{\text{recorded}}\) is the recorded amplitude of the ratio spectra of the laboratory prepared mixtures using 12 µg/ml CIN as a divisor at 250 nm and \(P_{\text{postulated}}\) is the calculated amplitude using the specified regression equation.

The original spectrum of CIN (X) in the mixture can be obtained by multiplying the obtained constant (CIN/CIN) of the laboratory mixtures by CIN (X) (the divisor), which is used for direct determination of CIN from the corresponding regression equation obtained.
by plotting the absorbance values of the zero order spectra at its 
\( \lambda_{\text{max}} \) 250 nm against the corresponding concentrations of CIN.

Component DIM (Y) can be determined by repeating the same steps using a spectrum of 10 \( \mu g/ml \) DIM as a divisor instead of CIN to calculate the constant value of DIM via amplitude difference step using regression equation.

\[
P_1 - P_2 = 0.9166P_1 - 0.0154 \quad r = 0.9999
\]

where \( P_1, P_2 \) are the ratio amplitudes at 250 nm and 270 nm of the ratio spectra of different concentration of CIN (4-20 \( \mu g/ml \)) using 10 \( \mu g/ml \) DIM as a divisor versus the corresponding ratio amplitudes at 250 nm as shown in (Fig. 8).

The original spectrum of DIM is obtained after multiplication of the calculated constant value by the spectrum of 10 \( \mu g/ml \) DIM as a divisor.

**Mean centring method (MCR)**

Mean centering method depends on the manipulation of the ratio spectra by the MATLAB software to cancel the effect of one component of the mixture to determine the other one. It eliminates the derivative step and therefore the signal-to-noise ratio is enhanced [22,23]. The absorption spectra of CIN (4–20 \( \mu g/ml \)) are divided by the spectrum of DIM 10 \( \mu g/ml \). The ratio spectra obtained in the range of (210–300 nm) are mean centered where the obtained mean centered spectra have peak maximum at 249 nm, (Fig. 9); then the calibration curve is constructed between the concentration of CIN and amplitude at 249 nm. The absorption spectra of DIM (10–45 \( \mu g/ml \)) are divided by the spectrum of zero order absorption spectrum of CIN (12 \( \mu g/ml \)) and the obtained ratio spectra in the range of (200–400 nm) is mean centered, where the obtained mean centered spectra have peak maximum at 280 nm, (Fig. 10); then the calibration curve is constructed between that amplitude and the corresponding concentration of DIM.

For all the proposed methods, the statistical parameters of the regression equations and the concentration ranges are shown in (Table 1). The selectivity of the proposed procedures is assessed by the analysis of laboratory prepared mixtures containing different ratios of the CIN and DIM, where satisfactory results are obtained over the calibration ranges as shown in (Table 2).

The proposed spectrophotometric methods were applied for the determination of CIN and DIM in their combined pharmaceutical formulation (Amocerebral plus tablets). The validity of the methods was assessed by applying the standard addition technique (Table 3). It shows that the developed methods are accurate and specific for determination of the cited drugs in presence of dosage form excipients.

**Statistical analysis**

Results of the suggested methods for determination of CIN and DIM are statistically compared with those obtained by applying a reported spectrophotometric method [14] (Table 4). The calculated
t- and F-values [24] are found to be less than the corresponding theoretical ones, confirming good accuracy and excellent precision.

**Conclusion**

The dual wavelength method depends on the zero order absorption spectrum and the overlapping problem is solved without any changes or manipulation in the original zero order absorption spectrum of the mixture. That is time and effort saving as well as it decreases the errors during manipulation steps and maintains maximum accuracy.

RD method has the advantages of being simpler and selective than the conventional spectrophotometric ones as it does not need critical measurement at fixed wavelengths or any derivative calculation, hence signal to noise ratio is enhanced and by difference between two wavelengths, noise will be cancelled.

The constant center method has the advantage of getting the zero order absorption spectra of both drugs which act as spectral profile of the drug subsequently the drugs are analyzed by using the absorbance value at their $\lambda_{\text{max}}$ which offer maximum accuracy and precision with minimum number of manipulation steps.

The mean centering method is also so simple and can be carried in very few steps using the MATLAB software giving accurate and precise methods.

The advantage of the constant center method over the other proposed methods as it predicts the UV spectra of each single drug without using any complicated software.

The developed methods can be used as alternative methods to LC methods in laboratories lacking the required facilities for these techniques for the analysis of any binary mixture without any limitation. They could be used for routine analysis of CIN and DIM in their available dosage form without any preliminary separation steps.

**References**