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Determination of dimenhydrinate and cinnarizine in combined dosage form in presence of cinnarizine impurity

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ABSTRACT

Three accurate, sensitive and time saving spectrophotometric methods have been developed and validated for determination of mixture of dimenhydrinate (DMH) and cinnarizine (CIN) in presence of cinnarizine impurity (1-(diphenylmethyl)piperazine) (IMP). In method A; dimenhydrinate was determined by measuring ^{1}D amplitudes at 292.0 nm while cinnarizine and its impurity were determined by ^{1}DD method at 256.2 and 219.6 nm, respectively, using standard spectrum of 20 µg/mL of dimenhydrinate as a divisor. Method B depends on dividing spectrum of ternary mixture by standard spectrum of 20 µg/mL of dimenhydrinate and then cinnarizine and its impurity were determined in the obtained ratio spectrum by ratio difference method using the difference between 219.0 and 237.2 nm and between 230.0 and 264.0 nm, respectively. On the other hand dimenhydrinate could be determined by dividing spectrum of ternary mixture by standard spectrum of 20 µg/mL of cinnarizine and then it were determined at the obtained ratio spectrum by ratio difference method using the difference between 216.8 and 232.8 nm. Method C is the mean cantering of ratio spectra method (MCR) where the amplitudes at 234.8, 240.0 and 233.6 nm in the second mean centering ratio spectra were used for determination of dimenhydrinate, cinnarizine and its impurity, respectively. The developed methods were validated according to ICH guidelines regarding good accuracy and precision, and they were successfully applied to pharmaceutical formulation and laboratory prepared mixtures. The results were statistically compared with those obtained by reported method and no significant difference was found.

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1. Introduction

Dimenhydrinate; is chemically known as 8-chloro-3,7-dihydro-1, 3-dimethyl-1*H*-purine-2,6-dione compound with 2-(diphenylmethoxy)-*N*,*N*-dimethylethanamine (1:1), it is the diphenhydramine salt of 8-chlorotheophylline [1] (Figure 1). DMH has antihistaminic with antimuscarinic and significant sedative effects. It is mainly used as an antiemetic drug in the prevention and treatment of motion sickness [2]. Cinnarizine; chemically known as (*E*)-1-(diphenylmethyl)-4-(3-phenylprop-2-enyl) piperazine [1] (Figure 1), it is a piperazine derivative with antihistaminic, sedative and calcium-channel blocking activity. It is used for the symptomatic treatment of nausea, vertigo, treatment of motion sickness and cerebral vascular disorders [2]. British Pharmacopeia [3] stated that 1-(diphenyl methyl) piperazine is cinnarizine impurity, it is also known as benzhydrylpiperazine, [1] (Figure 1).

Cinnarizine is official in British Pharmacopeia (BP) [3] and European Pharmacopeia (EP) [4], both of them includes potentiometric titration for estimation of CIN. Dimenhydrinate is official in BP [3], EP [4] and United States Pharmacopeia (USP) [5], which includes potentiometric titration, argentometric titration and HPLC method for estimation of DMH. The combination of these two drugs is not official in any pharmacopoeia.

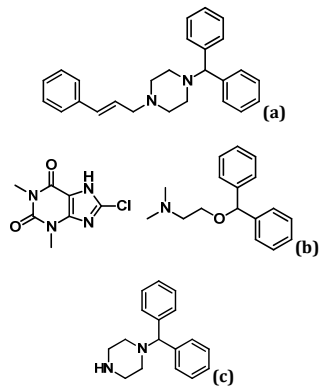

Figure 1. Chemical structures of (a) cinnarizine, (b) dimenhydrinate, (c) diphenylmethyl piperazine (impurity).

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Literature review shows that numbers of analytical methods are available for estimation of both the drugs either spectrophotometry including: Q-absorbance ratio spectrophotometric method [6], simultaneous chemical equation method [7], derivative spectrophotometric method [8] and dual wavelength spectrophotometric method [9], or chromatography including: validated HPTLC method [10] and HPLC-method [11,12].

We have not come across any analytical methods for determination of both the drugs in presence of cinnarizine impurity. Either of these methods can be used for determination of cinnarizine impurity. Therefore, the objective of work is to develop three accurate, sensitive and reliable spectrophotometric method for determination of cinnarizine and dimenhydrinate in presence of cinnarizine impurity with high sensitivity and selectively and to validate the developed methods according to ICH guidelines [13].

2. Experimental

2.1. Instrumentations

A double beam UV-visible spectrophotometer, Shimadzu, Japan, Model UV-1601 PC with quartz cell of 1 cm and UV-PC personal software version 3.7 was used. The spectral band width is 2 nm and wavelength-scanning speed 2800 nm/min. Data analysis for mean centering of ratio spectra method was performed using PLS-Toolbox 2.0 running under MATLAB®, version 6.5.

2.2. Materials

2.2.1. Pure samples

Pure samples of CIN and DMH were kindly supplied by Amoun Pharmaceutical Company, Cairo, Egypt with claimed purity of 99.6 and 99.7%, respectively, according to manufacturer certificates of analysis. While cinnarizine impurity (IMP) was obtained from (Sigma-Aldrich Chemie GmbH, Germany) with claimed purity of 97% according to the manufacturing certificates of analysis.

2.2.2. Pharmaceutical dosage form

Amocerebral® tablets (Batch No. 6221025030658) were manufactured by Amoun Pharmaceutical Company, Cairo, Egypt. Each tablet is claimed to contain 10 mg of CIN and 20 mg of DMH.

2.2.3. Solvents

Methanol HPLC grade (Chromasolve®, Sigma-Aldrich Chemie GmbH, Germany).

2.2.4. Solutions

Stock solutions of CIN, DMH and IMP were prepared in methanol in the concentration of 1 mg/mL. They were prepared by weight 0.1 g of each drug in three separated 100 mL calibrated flasks. Working solutions of CIN, DMH and IMP were prepared in methanol in the concentration of 100 µg/mL by separately diluting 5 mL of each stock solution in three separated 50 mL volumetric flask.

3. Methods

3.1. Linearity

Accurate aliquots equivalent to 30-300, 20-400 and 20-200 µg from CIN, DMH and IMP, respectively, were taken from their working solutions (100 µg/mL) and transferred into three separate series of 10 mL volumetric flasks then completed to the mark with methanol. Zero order absorption spectra of the prepared samples were measured in the range of 200-400 nm (Figure 2).

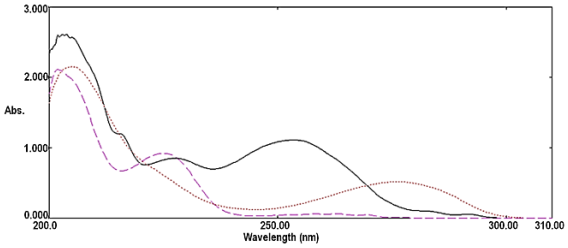

Figure 2. Zero order absorption spectra of 20 µg/mL of each of cinnarizine (——), dimenhydrinate (…) and impurity (---) using methanol as a blank.

3.1.1. Method A

Calibration curve of DMH was constructed by plotting ^{1}D amplitudes at 292.0 nm (Δλ = 2 and scaling factor = 10) versus the corresponding concentration in the range of 2-40 µg/mL. For determination of CIN and IMP, the spectra of the prepared samples in the range of 3-30 and 2-20 µg/mL, respectively, were divided by standard spectrum of 20 µg/mL of DMH, then first derivative of the obtained ratio spectra were obtained using Δλ = 2 and scaling factor = 10. 1DD amplitudes at 256.2 and 219.6 nm were obtained from which calibration graphs for CIN and IMP, respectively, were obtained and the regression equations were computed.

3.1.2. Method B

For determination of DMH, its absorption spectra were separately divided by the standard spectrum of 20 µg/mL of CIN, then DMH was determined by measuring the ratio difference at 216.8 and 232.8 nm (at which CIN and IMP have zero difference). While for determination of CIN and IMP their absorption spectra were separately divided by the standard spectrum of 20 µg/mL of DMH, then CIN was determined by measuring the ratio difference at 219.0 and 237.2 nm (at which DMH and IMP have zero difference). While IMP was determined by measuring the ratio difference between 230.0 and 264.0 nm (zero difference for both DMH and CIN). The calibration curves for DMH, CIN and IMP were constructed by plotting the ratio difference (for DMH, CIN and IMP) at the selected wavelengths against their corresponding concentrations and the regression equations were obtained.

3.1.3. Method C (Mean centering of ratio spectra spectrophotometric method)

The stored spectra of three components were exported to Matlab program for subsequent calculation. For determination of CIN; the recorded spectra of CIN were divided by the standard spectrum of DMH (20 µg/mL) to obtain the first ratio spectra which were then mean centered. These vectors were then divided by the mean centered ratio of αIMP/αDMH (corresponding to standard spectrum of 20 µg/mL each) and the mean center of the second ratio spectra was then obtained.

By the same way, the recorded spectra of DMH were divided by the standard spectrum of 20 µg/mL of IMP and the obtained ratio spectra were then mean centered, these vectors (the mean centered ratio spectra) were divided by the mean centered ratio of αCIN/αIMP (corresponding to standard spectrum of 20 µg/mL each) to obtain the second ratio spectra which then mean centered.

For determination of the IMP, the scanned spectra of its prepared solutions were divided by the standard spectrum of 20 µg/mL of DMH and the obtained ratio spectra were then

mean centered. These vectors were divided by the mean centered ratio of αDMH /αCIN (corresponding to standard spectrum of 20 µg/mL each) and the second ratio spectra were then mean centered. The amplitudes of the second mean centered ratio spectra at 240.0, 234.8 and 233.6 nm were used for determination of CIN, DMH and IMP, respectively, using the corresponding regression equations.

3.2. Analysis of laboratory prepared mixtures

Mixtures containing different ratios of CIN, DMH and IMP (in the ratio of 10-80% of CIN) were prepared. The volume was completed with methanol. The procedure under linearity for each method was then followed and the concentration of each component was calculated from the corresponding regression equations.

3.3. Application to pharmaceutical formulation (Amocerebral® tablets)

Ten tablets of Amocerebral® tablets were weighed, powdered and mixed well; an accurate weight of the powdered tablets equivalent to 100 mg of CIN and 200 mg of DMH was transferred into 100 mL volumetric flask. 75 mL of methanol was added and sonicated for 20 min; then filtered into 100 mL volumetric flask. The residue was washed using methanol then the volume was completed with methanol to obtain stock solution of 1 mg/mL. From which sample working solution of 100 µg/mL was prepared and then the procedure under linearity for each method was followed and concentrations of DMH and CIN were calculated using the previously computed regression equation.

Standard addition has been carried out to assess validity of the method by spiking the pharmaceutical formulation by known amount of standard drug powder. The recovery of the added standards was then calculated after applying the proposed methods.

4. Results and discussion

Different analytical methods have been found in the literature for analysis of DMH and CIN binary mixtures. But these methods cannot be applied for resolving the ternary mixture of DMH, CIN and IMP. As previously mentioned no published method was found in the literature for determination of DMH and CIN in presence of CIN impurity. The work in this manuscript concerned with the development and validation of three accurate, selective and precise spectrophotometric methods for resolving the overlapped spectra of DMH, CIN and IMP (Figure 2) with minimum sample preparation and data manipulation.

4.1. Method development and validation

There are many factors that affect selectivity and sensitivity of the developed methods such as: the used solvent, the divisor concentration and the chosen wavelength. Different solvents were tried such as methanol, ethanol, acetonitrile, water, 0.1 N HCl and 0.1 N NaOH. Regarding method selectivity, methanol was the solvent of choice.

Since the divisor and its concentration are greatly effect method selectivity, so different divisors with different concentrations were tried using of standard spectrum of 20 µg/mL DMH for method A, B and C; 20 µg/mL CIN for method B, C and 20 µg/mL IMP for method C gave the best results.

Selections of wavelengths play important rule during development and optimization of method B. Different wavelength pairs were tested where the wavelength pairs of 216.8 and 232.8 nm, 219.0 and 237.4 nm and 230.0 and 264.0 nm were the pairs of chosen for determination of DMH, CIN and IMP, respectively.

4.1.1. Method A

In this method, DMH could be determined by first derivative (^{1}D) spectrophotometric method using Δλ = 2 nm and scaling factor = 10 then measuring the peak amplitude values at 292 nm, Figure 3. On the other hand, the absorption spectra of different concentrations of CIN and IMP were recorded in the wavelength range of 200-400 nm. The recorded spectra were divided by the standard spectrum of 20 µg/mL DMH and the ratio spectra were obtained, then first derivative of these ratio spectra were obtained using Δλ = 2 nm and scaling factor = 10 and peak amplitudes were measured at 256.2 and 219.6 nm for CIN and IMP, respectively, (Figure 4).

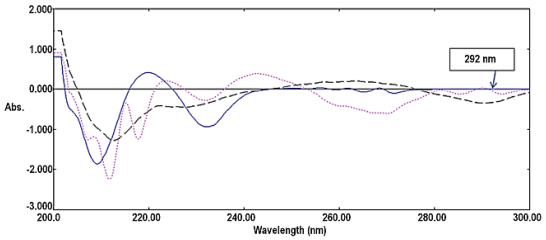

Figure 3. First derivative spectra of 20 µg/mL of each of cinnarizine (...), dimenhydrinate (---) and impurity (—) using methanol as a blank.

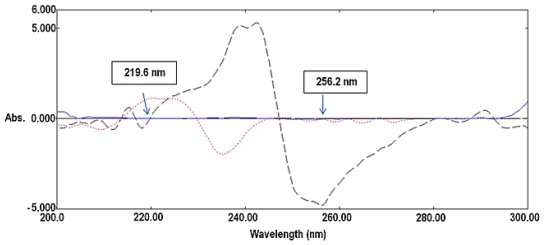

Figure 4. First derivative of ratio spectra of 20 µg/mL of each cinnarizine (---), dimenhydrinate (—) and impurity (...) using standard spectrum of 20 µg/mL of dimenhydrinate as a divisor and methanol as a blank.

Calibration curves relating the peak amplitude values at the selected wavelength versus the corresponding concentration of DMH, CIN and IMP in the concentration range of 2-40, 3-30, 2-20 µg/mL, respectively, from which the following regression equations were computed, Table 1.

$$Y1 = 0.0166 \times C_{[DMH]} + 0.0045, r = 0.9999 \quad (1)$$

$$Y2 = 0.2361 \times C_{[CIN]} + 0.1112, r = 0.9999 \quad (2)$$

$$Y3 = 0.0540 \times C_{[IMP]} + 0.0260, r = 0.9999 \quad (3)$$

where Y1, Y2 and Y3 are peak amplitudes for DMH, CIN and IMP, respectively, at selected wavelengths, C is the concentration in µg/mL and *r* is the correlation coefficient.

4.1.2. Method B

This method depended on using the ratio difference method which has the same principle as dual wavelength spectrophotometric method. The principle of this method is that the ratio difference at two wavelengths in the ratio spectra is directly proportional to the concentration of the component of interest where the other components show zero difference at the selected wavelengths. Ratio difference method has advantages of being able to resolve ternary and quaternary mixtures and also it eliminate derivative steps and so signal to noise ratio is enhanced.

Table 1. Assay parameters and method validation for the determination of pure sample of CIN, DMH and IMP by the proposed spectrophotometric methods.

Parameter	CIN	DMH	IMP
Method A			
Calibration range (µg/mL)	3-30	2-40	2-20
Slope	0.2361	0.0166	0.0540
Intercept	0.0111	0.0045	0.0260
Correlation coefficient	0.9999	0.9999	0.9999
Accuracy (Mean±SD)	100.21±0.864	100.05±0.457	100.27±0.670
Precision			
Repeatability [a]	1.74	1.64	1.25
Intermediate precision [b]	1.36	1.84	1.70
Specificity	100.70±0.953	100.30±1.764	99.90±1.331
LOD [c] (µg/mL)	0.93	0.48	0.42
LOQ [c] (µg/mL)	2.85	1.47	1.27
Method B			
Calibration range (µg/mL)	3-30	2-40	2-20
Slope	0.1525	0.0453	0.0366
Intercept	0.0592	0.0955	0.0060
Correlation coefficient	0.9999	0.9998	0.9998
Accuracy (Mean±SD)	100.06±1.217	99.98±0.771	99.92±0.903
Precision			
Repeatability [a]	0.89	1.58	1.14
Intermediate precision [b]	1.03	1.08	1.67
Specificity	100.49±0.919	100.09±1.076	99.90±0.863
LOD [c] (µg/mL)	0.95	0.58	0.48
LOQ [c] (µg/mL)	2.91	1.87	1.65
Method C			
Calibration range (µg/mL)	3-30	2-40	2-20
Slope	1.2824	3.9573	8.4036
Intercept	0.0269	4.5744	2.5860
Correlation coefficient	0.9999	0.9998	0.9998
Accuracy (Mean±SD)	100.05±0.577	100. 17±1.315	101.17±1.326
Precision			
Repeatability [a]	1.85	1.05	0.97
Intermediate precision [b]	0.92	1.22	1.10
Specificity	100.12±1.237	99.60±1.404	100.14±1.177
LOD [c] (µg/mL)	0.87	0.46	0.60
LOQ [c] (µg/mL)	2.74	1.45	1.90

[a] The intraday (n=3), average of three different concentrations repeated three times daily.
[b] The interday (n=3), average of three different concentrations repeated three times in three successive days.
[c] Limit of detection (3.3×SD/Slope) and limit of quantitation (10×SD/Slope).

Choosing of wavelength pairs play important rule in method selectivity, hence different pairs were tested. Measuring the difference between 216.8-232.8 nm, 219.0-237.2 nm and 230.0-264.0 nm gave the best results.

The absorption spectra of DMH, CIN and IMP were separately divided by standard spectrum of 20 µg/mL CIN (Figure 5). So DMH was determined using the ratio difference between 216.8-232.8 nm in the obtained ratio spectra. On the other hand The absorption spectra of CIN, DMH and IMP were separately divided by standard spectrum of 20 µg/mL DMH (Figure 6). So, there were determined using the ratio difference between 219.0-237.2 nm and 230.0-264.0 nm, respectively, in the obtained ratio spectra, Figure 6.

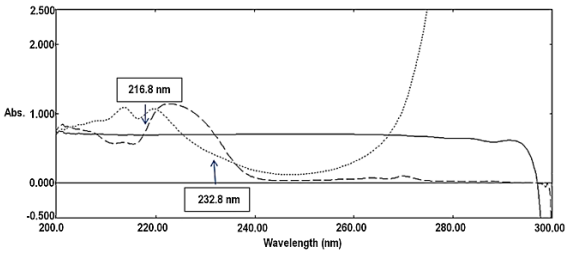

Figure 5. Division spectra of 20 µg/mL of each of cinnarizine (—), dimenhydrinate (...) and impurity (---) using standard spectrum of 20 µg/mL of cinnarizine as a divisor and methanol as a blank.

Calibration graphs were obtained by plotting the amplitudes difference at the selected wavelengths versus the corresponding concentrations from which regression equations were computed and found to be:

$$Y1 = 0.0272 \times C_{[DMH]} + 0.0034, r = 0.9999 \quad (4)$$

$$Y2 = 0.1525 \times C_{[CIN]} + 0.0592, r = 0.9999 \quad (5)$$

$$Y3 = 0.0366 \times C_{[IMP]} + 0.0060, r = 0.9998 \quad (6)$$

Where Y1, Y2 and Y3 are the amplitudes values or difference values at the selected wavelengths, C is the concentration in µg/mL and *r* is the correlation coefficient.

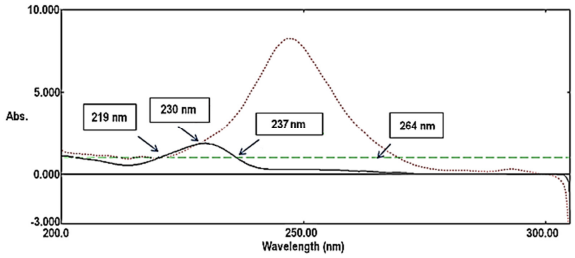

Figure 6. Division spectra of 20 µg/mL of each of cinnarizine (...), dimenhydrinate (---) and impurity (—) using standard spectrum of 20 µg/mL of dimenhydrinate as a divisor and methanol as a blank.

4.1.3. Method C

The proposed MCR method is based on the mean centering of ratio spectra. This method was applied for resolving binary and ternary mixtures in the complex samples with unknown matrices and it eliminates signal to noise ratio and derivatization step. The effect of divisor concentration on the selectivity of this method has been tested in order to optimize the developed method.

Different concentrations of CIN, DMH and IMP were tested. It was found that 20 µg/mL of DMH, CIN and IMP were the suitable divisors.

Table 2. Assay result for the determination of CIN, DMH and IMP in synthetic mixtures using the proposed spectrophotometric methods.

Ratio of CIN:DMH	% Impurity	Concentration taken (µg/mL)			% Recovery		
		CIN	DMH	IMP	CIN	DMH	IMP
Method A							
3:1	10	27	10	3	100.97	101.25	98.64
1:2	30	14	40	6	99.84	101.51	100.04
1:1	40	9	30	6	101.70	98.06	99.69
1:1	50	10	20	10	101.35	101.59	101.30
1:1	70	6	20	14	101.12	101.41	101.50
1:2	80	4	10	16	99.24	98.00	98.26
Mean±SD					100.14±1.177	99.60±1.404	100.12±1.237
Method B							
3:1	10	27	10	3	99.36	101.36	100.26
1:2	30	14	40	6	101.21	99.54	101.11
1:1	40	9	30	6	99.83	99.31	98.66
1:1	50	10	20	10	99.99	99.45	99.26
1:1	70	6	20	14	100.78	101.59	99.80
1:2	80	4	10	16	101.78	99.31	100.32
Mean±SD					99.90±0.863	100.09±1.076	100.49±0.919
Method C							
3:1	10	27	10	3	100.39	98.13	100.38
1:2	30	14	40	6	99.63	98.26	98.68
1:1	40	9	30	6	101.06	99.51	99.39
1:1	50	10	20	10	101.87	99.23	101.28
1:1	70	6	20	14	99.34	100.83	101.68
1:2	80	4	10	16	98.45	101.66	99.43
Mean ± SD					99.90±1.331	100.30±1.764	100.70±0.953

For determination of CIN, the recorded spectra of CIN were divided by the standard spectrum of 20 µg/mL DMH to obtain the first ratio spectra which were then mean centered. These vectors were then divided by the mean centered ratio of αDMH /αIMP (corresponding to standard spectrum of 20 µg/mL each) and the mean centering of the second ratio spectra was then obtained. By the same way, the recorded spectra of DMH were divided by the standard spectrum of IMP (20 µg/mL) and the obtained ratio spectra were then mean centered, these vectors (the mean centered ratio spectra) were then divided by the mean centered ratio of αIMP/αCIN (corresponding to standard spectrum of 20 µg/mL each) to obtain the second ratio spectra which then mean centered. For determination of IMP, the scanned spectra of its prepared solutions were divided by the standard spectrum of 20 µg/mL of DMH and the obtained spectra were then mean centered. These vectors were divided by the mean centered ratio of αDMH/αCIN (corresponding to standard spectrum of 20 µg/mL each) and the second spectra ratio were then mean centered. The amplitudes of the second mean centered ratio spectra at 240.0, 234.8 and 233.6 nm were used for determination of CIN, DMH and IMP, respectively. The amplitudes were measured and plotted against the corresponding concentration of each component to compute their respective regression equations (Figure 7-9). Linearity of the proposed method was evaluated and it was evident in the range of 3-30, 2-40 and 2-20 µg/mL for CIN, DMH and IMP, respectively. The regression equations for each component were calculated as given in Table 1 and found to be:

$$Y1 = 3.9573 \times C_{[DMH]} - 4.5744, r = 0.9998 \quad (7)$$

$$Y2 = 1.2824 \times C_{[CIN]} + 0.2696, r = 0.9999 \quad (8)$$

$$Y3 = 8.4036 \times C_{[IMP]} - 2.5860, r = 0.9998 \quad (9)$$

where Y1, Y2 and Y3 are the peak amplitudes value at the selected wavelengths, C is the concentration in µg/mL and *r* is the correlation coefficient.

The selectivity of the proposed methods was evaluated by analysis of different laboratory prepared mixtures containing different ratios of the studied components, where the mean percentage recoveries for DMH, CIN and IMP were 99.60±1.404, 100.14±1.177 and 100.12±1.237, respectively, for method A, 100.09±1.076, 99.90±0.863 and 100.49±0.919, respectively, for method B and 100.30±1.764, 99.90±1.331 and 100.70±0.953, respectively, for method C, Table 2.

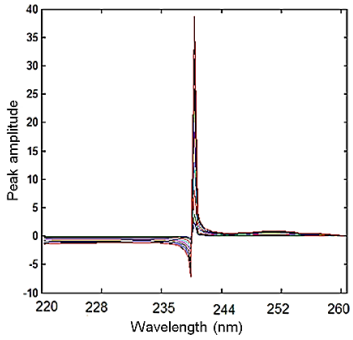

Figure 7. Mean centered ratio spectra of cinnarizine (3-30 µg/mL) using methanol as a blank.

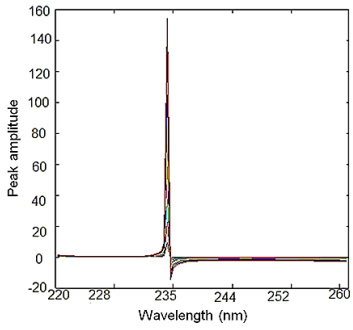

Figure 8. Mean centered ratio spectra of dimenhydrinate (2-40 µg/mL) using methanol as a blank.

The developed spectrophotometric methods were also applied for determination of CIN and DMH in Amocerebral® tablets and satisfactory results were obtained (Table 3). The validity of the methods was further assessed by applying standard addition technique which also confirmed the accuracy of the proposed methods (Table 3). The results obtained by applying the proposed methods were statistically compared with those obtained by applying the reported HPLC method for determination of Amocerebral® tablets.

Table 3. Determination of CIN and DMH in their pharmaceutical formulation by the proposed spectrophotometric methods and application of standard addition technique.

Pharmaceutical formulation	Methods	Added (µg/mL)		Recovery, %		Found %	
		CIN	DMH	CIN	DMH	CIN	DMH
Amocerebral plus ® tablets	Method A	5	5	100.78	99.39	103.14±1.079	105.77±1.601
CIN, 10 mg		7	10	99.34	101.50		
DMH, 20 mg		10	15	101.34	98.59		
		15	20	101.22	100.75		
		Mean ± SD		100.67±0.921	100.06±1.311		
	Method B	5	5	98.07	101.32	104.27±1.628	103.79±1.396
		7	10	99.74	100.99		
		10	15	101.88	98.96		
		15	20	101.89	100.16		
		Mean ± SD		100.40±1.852	100.36±1.049		
	Method C	5	5	100.95	101.63	104.46±1.418	104.67±1.284
		7	10	99.36	101.19		
		10	15	100.02	98.27		
		15	20	101.87	99.49		
		Mean ± SD		100.55±1.098	100.152±1.551		

Table 4. Statistical comparison of the results obtained by applying the proposed spectrophotometric methods and the reported methods for analysis of CIN and DMH in their pharmaceutical formulation.

Parameter	Method A		Method B		Method C		Reported method [12]	
	CIN	DMH	CIN	DMH	CIN	DMH	CIN	DMH
Mean	103.14	105.77	104.27	103.79	104.46	104.67	106.16	103.72
SD	1.079	1.601	1.628	1.396	1.418	1.284	1.747	1.783
Variance	1.164	2.564	3.183	2.340	2.011	1.648	3.664	3.815
N	6	6	6	6	6	6	6	6
t-test [a]	1.8595	1.9861	1.7696	0.6133	1.7425	0.9936	-	-
F-value [a]	3.1475	0.6720	1.1513	0.0700	1.8216	0.4321	-	-

[a] The values in parenthesis are corresponding to the theoretical values of *t* and *F* (p = 0.05).

The values of the obtained *F* and *t*-tests were less than the calculated ones confirming that the difference between the developed methods and the reported one is non-significant, Table 4.

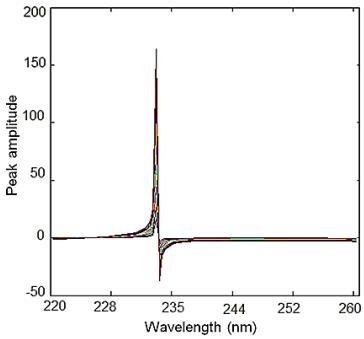

Figure 9. Mean centered ratio spectra of impurity (2-20 µg/mL) using methanol as a blank.

4.2. Method validation

Validation of the methods was carried out according to ICH recommendations [13].

4.2.1. Linearity and range

The linearity of the proposed methods was evaluated by analyzing different concentrations of CIN, DMH and IMP in triplicates. For the developed methods, Beer-Lambert's was obeyed in the range of 3-30, 2-40 and 2-20 µg/mL for CIN, DMH and IMP, respectively. The values of correlation coefficients were close to unity indicating good linearity. The regression parameters like the slope, intercept and the correlation coefficient were calculated and are presented in Table 1.

4.2.2. Accuracy

Accuracy of the proposed methods was calculated as the percentage recoveries of pure samples of the studied drugs. The concentrations were calculated from the corresponding regression equations, Table 1. Accuracy was further assessed by applying the standard addition technique to Amocerebral® tablets, where good recoveries were obtained revealing that there was no interference from excipients, Table 3.

4.2.3. Precision

Repeatability: Three concentrations 7, 15, 25 µg/mL of CIN; 20, 30, 40 µg/mL of DMH and 7, 12, 20 µg/mL of IMP were analyzed three times intra-daily using the proposed methods. Good results and acceptable standard deviations (SDs) were obtained, Table 1.

Intermediate precision: The previous methods were repeated inter-daily on three different days for the analysis of the chosen concentrations. Good results and acceptable SDs were obtained; good results were obtained and presented in Table 1.

4.2.4. Selectivity

Selectivity of the proposed methods was assessed by the analysis of different synthetic laboratory prepared mixtures containing different ratios of CIN, DMH and IMP within their linearity ranges; good results were obtained and presented in Table 1.

4.2.5. Detection and quantitation limits

These approaches based on the SD of the response and the slope were used for determining the detection and quantitation limits where LOD = 3.3×SD/slope and LOQ = 10×SD/slope, Table 1.

5. Conclusion

The developed methods have advantages over the published methods in being used for analysis of cinnarizine and dimenhydrinate in the presence of cinnarizine impurity. These methods were simple, rapid, and cost effective and data processing steps are not time consuming. These spectrophotometric methods can be regarded as a useful alternative to chromatographic techniques in the routine quality control analysis of pharmaceutical formulations allowing rapid determination at relatively low cost. The developed methods can be easily and conveniently adopted for routine quality control analysis of CIN, DMH and IMP.

References

[1]. Budavaried, S. The Merck Index: An encyclopedia of chemicals, drugs and biological, 40th Ed., Merck & Co., Inc., Whitehouse Station, NJ, USA, 2006.
[2]. Profitt, K. Martindale, The extra Pharmacopeia: The complete drug reference, 35th Ed., Royal Pharmaceutical Society, London, UK, 2006.
[3]. British Pharmacopoeia, Vol. II, Stationary Office, Medicines and Health care Products Regulatory Agency, London, UK, 2010.
[4]. European Pharmacopoeia, 6th Edi., European Department for the Quality of Medicines within the Council of Europe, Strasbourg, France, 2008
[5]. The United States Pharmacopoeia, 32th revision, NF 27, The United States Pharmacopoeial Convention Inc., Rockville, MD, 2009.
[6]. Shah, P.B.; Patel, P. U. *J. Pharm. Sci. Bio-Sci. Res.* **2012**, *2(2)*, 83-87.
[7]. Dalwadi, N.; Chhalotiya, U. K.; Shah, D.; Mehta, F.; Bhatt, K. *Int. J. Adv. Pharm. Anal.* **2014**, *4(2)*, 58-61.
[8]. Patel, A. P.; Kadikar, H. K.; Shah, R. R.; Shukla, M. H.; Tank, P. K. *Pharma Sci. Mon. Int. J. Pharm. Sci.* **2012**, *3(4)*, 2493-2505.
[9]. Lamie, N. T. *Spectrochim. Acta A* **2015**, *141*, 193-201.
[10]. El-Kafrawy, D. S.; Belal, T. S. *J. Assoc. Arab Univ. Basic Appl. Sci.* **2014**, doi:10.1016/j.jaubas.2014.06.004.
[11]. Khushbu, S.; Pinkal, P. *Int. J. Pharm. Sci. Res.* **2014**, *5(11)*, 4815-4819.
[12]. Lamie, N. T.; Monir, H. H. *J. Chromatogr. Sci.* **2015**, doi:10.1093/chromsci/bmv103
[13]. International conference on the harmonization of technical requirements for the registration of pharmaceuticals for human use (ICH) Q2 (R1) Validation of Analytical Procedures: Text and Methodology. ICH Harmonized Tripartite Guideline, 2005.