Development and Validation of Spectrophotometric Methods for the Simultaneous Determination of Mebeverine Hydrochloride and Chlordiazepoxide in Bulk and in Dosage Form

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Abstract

Two accurate, precise and simple spectrophotometric methods had been developed and validated for the determination of mebeverine hydrochloride (MEB) and chlordiazepoxide (CHL). The first method was used for the determination of MEB in the range of 5-81 µg/mL and CHL in the range of 0.5-4 µg/mL, MEB was determined by second derivative spectrophotometric determination by measuring the peak amplitude at 234.8 nm (zero value of CHL) whereas CHL was determined by first derivative spectrophotometric method by measuring the peak amplitude at 280.4 nm (zero value of MEB). In the second method, three chemometric methods were evaluated for simultaneous assay of MEB and CHL. The developed methods were successfully applied to the analysis of the cited drugs in laboratory prepared mixtures and tablets with good recoveries. The validity of the proposed methods was further assessed by applying the standard addition technique. The validation was carried out following the International Conference on Harmonization (ICH) guidelines.

Keywords: Mebeverine Hydrochloride; Chlordiazepoxide; Derivative spectrometry; Chemometrics techniques

Introduction

Mebeverine hydrochloride is 3,4-dimethoxybenzoic acid 4-[ethyl[2-(4-methoxy phenyl)-1-methylethyl]amino]-butylester hydrochloride (Figure 1). It is the most prescribed product currently available for the treatment of irritable bowel syndrome (IBS). It acts as a musculotropic antispasmodic agent with a direct action on the smooth muscle of the gastrointestinal tract especially of the colon, revealing spasm without affecting normal gut motility, and possessing no atropine like action [1-3].
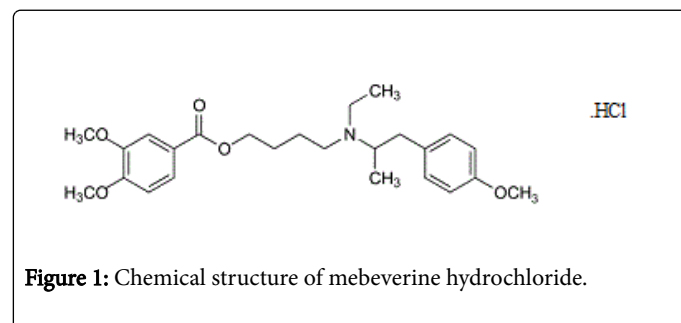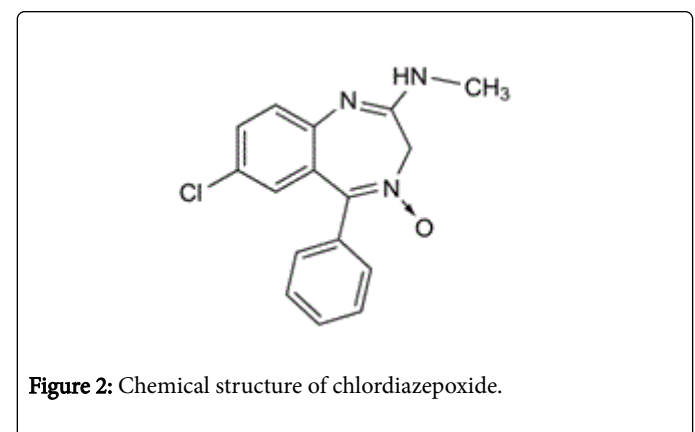

Figure 1: Chemical structure of mebeverine hydrochloride.

Chlordiazepoxide is 7-chloro-N-methyl–5–phenyl-3H-1,4–benzodiazepine–2–amine 4–oxide (Figure 2). It is a benzodiazepine mainly used in IBS to enhance the effect of the neurotransmitter gamma-amino butyric acid (GABA) at the GABAA receptor, resulting in sedative, hypnotic (sleep-inducing), anxiolytic (anti-anxiety), anticonvulsant and muscle relaxant properties [4].

Figure 2: Chemical structure of chlordiazepoxide.

A literature survey has revealed several analytical methods for the determination of MEB in pharmaceutical dosage form, including spectrophotometry [5-9] conductometry, spectroscopy [10] and liquid chromatography [11-20]. Only few spectrophotometric methods have been reported for the simultaneous estimation of MEB and CHL in capsules in different pH's [21-22], and in tablet form using simultaneous equations and by Q-analysis method has been developed [23]. Univariate spectrophotometric methods via different manipulation pathways [24] has been reported for the simultaneous determination of MEB and CHL. Besides, MEB and CHL has been determined by HPLC [25-29].

A literature survey has revealed several analytical methods for the determination of CHL either alone or in mixtures with other drugs [30-40].

Thus the aim of the present work is to develop and validate simple, rapid and inexpensive analytical methods using the derivative spectrophotometry and chemometric techniques for the simultaneous determination of MEB and CHL to be applied to their determination in pharmaceutical preparation as a substitute for the published methods for the cited mixture.

Derivative spectrophotometry is an analytical technique of great utility for extracting of both qualitative and quantitative information from spectra composed of unresolved bands and for eliminating effect of baseline shifts. It also allows fast resolution of multicomponent mixture of drugs [41]. Alternatively, in recent years multivariate calibrations, such as Classical Least Squares (CLS), Principal Component Regression (PCR), and Partial Least Squares (PLS) started to be applied to the analysis of the analytical data obtained in all the instrumentations. The same methods have been applied to the simultaneous spectrophotometric determination of drugs in pharmaceutical formulations containing two or more compounds with overlapping spectra [42,43]. Besides, the chemometric calibration methods have been used extensively in quantitative spectral analysis to get selective information from unselective data.

Experimental

Instrumentation

A Shimadzu 1800 double beam ultraviolet/visible spectrometer, Japan connected to an IBM compatible computer with 1 cm quartz cell supported with UV probe software was used.

Reagents and reference samples

Pharmaceutical grade MEB, certified to contain 99.80%, pharmaceutical grade CHL, certified to contain 99.80%. Coloverin A tablets nominally containing 135 mg MEB and 5 mg CHL per tablet were supplied from Chemipharm, Egypt. Methanol HPLC grade was purchased from Fisher Scientific, UK.

Preparation of standard and working solutions

Preparation of MEB standard stock solution: Accurately weight 25 mg MEB was transferred into a 25 mL volumetric flask and dissolved in and completed to the mark with methanol (1 mg/mL).

Preparation of MEB working solution: Five microliters of MEB stock solution were transferred into a 50 mL volumetric flask; the volume was completed to mark with methanol (100 µg/mL).

Preparation of CHL stock solution: Accurately weight 25 mg of CHL were transferred into a 25 mL volumetric flask and dissolved in and completed to volume with methanol (1 mg/mL).

Preparation of CHL working solution: Five microliters of CHL stock solution were transferred to a 50 mL volumetric flask and completed to mark with methanol (100 µg/mL), and 5mL of this solution were transferred into a 50 mL volumetric flask; the volume was completed to mark with methanol (10 µg/mL).

Procedure

Method I (Derivative spectrophotometric method) Construction of calibration curves: Different aliquots from MEB and CHL working solutions were transferred into two series of 10 mL volumetric flasks, the volumes were completed with methanol to give final concentrations of 5-81 µg/mL and 0.5-4 µg/mL for MEB and CHL, respectively.

The absorbance spectra of each solution was recorded against methanol as a blank in the range 200-400 nm, and then the second derivative spectra, and the first derivative for MEB and CHL respectively using Δλ=4 and a scaling factor=50 was computed. The amplitudes at 234.8 nm and 280.4 nm for MEB and CHL, respectively, were measured and then plotted against corresponding concentrations to obtain calibration graphs and the regression equations.

Method II (Chemometric method) Construction of Training set: Twelve binary mixtures of MEB and CHL were prepared by placing different volumes of their working solutions into series of 10 mL volumetric flasks the volumes were completed with methanol in table (1). The absorbances of these mixtures were measured between 260 and 310 nm at 0.2 nm intervals against methanol as a blank.

Construction of CLS, PCR, and PLS models: Three multivariate calibration models (CLS, PCR and PLS) were constructed using the data obtained. In these methods the absorbance data matrix for training set concentration matrix was obtained by measuring the absorbances between 260 and 310 nm at 0.2 nm intervals. Calibration or regression was then obtained by using the absorbance data matrix and the concentration data matrix for the determination of the unknown concentration of MEB/CHL in their binary mixtures and pharmaceutical dosage forms. For the CLS method, the model was constructed with non-zero intercept.

The computer was fed with the absorbance and concentration matrices of the training set and calculations were made to obtain the K matrix (i.e., absorptivity at different wavelength). For the PCR and PLS methods, the training set absorbance and concentration matrices together with PLS-toolbox 2.0 software were used for calculations.

Selection of the optimum number of factors to build the PCR and PLS models: In order to select the optimum number of factors, cross-validation method was used leaving out one sample at a time [44]. Given a set of 12 calibration spectra for MEB/CHL mixture, the PCR and PLS calibrations were performed and using this calibration the concentration of the sample left out was predicted. This process was repeated until each sample had been left out once.

The predicted concentrations of the components in each sample were compared with the actual concentrations in this calibration samples and Root-Mean-Square Error of Cross-Validation (RMSECV) was calculated for each method. It indicates both of the precision and accuracy of predictions. Visual inspection was used for deciding the optimum number of factors.

$$RMSECV = \sqrt{\frac{PRESS}{n}} \quad (1)$$

Where PRESS is the predicted residual error sum of squares and n is the number of calibration samples [45].

$$PRESS = \sum (Y_{pred} - Y_{true})^2 \quad (2)$$

Where Y pred and Y true are predicted and true concentrations in µg/mL, respectively

Sample No.	Claimed conc. (mg/mL)	
	MEB	**CHL**
1	27	0.8
2	27	1
3	27	1.2
4	35.1	1.1
5	35.1	1.3
6	35.1	1.5
7	51.3	1.7
8	51.3	1.9
9	51.3	2.1
10	59.4	2
11	59.4	2.2
12	59.4	2.4

Table 1: The concentrations of different mixtures of MEB and CHL used in the training set.

Assay of laboratory-prepared mixtures

Method I: For validation of derivative method, aliquots of MEB and CHL working solutions were accurately transferred into a series of 10 mL volumetric flasks to give final concentrations 18.9-51.3 µg/mL for MEB and 0.7-1.9 µg/mL for CHL as shown in Table 2.

The absorption spectrum was recorded for the laboratory prepared mixtures against methanol as a blank. The amplitudes of the second derivative spectra and first derivative spectra were measured at 234.8 nm for MEB and 280.4 nm for CHL, respectively. The concentrations of MEB and CHL were calculated from their corresponding regression equations.

Amount taken (µg/mL)		MEB		CHL	
MEB	CHL	Amount found (µg/mL)	% Recovery	Amount found (µg/mL)	% Recovery
18.9	0.7	18.9313	100.165	0.6926	98.94
21.6	0.8	21.6069	100.032	0.8055	100.69
24.3	0.9	24.1346	99.319	0.8964	99.596
29	1.1	29.0792	100.273	1.1117	101.06
35.1	1.3	34.9918	99.692	1.3002	100.02
45	1.6	44.962	99.916	1.591	99.45
45.9	1.7	45.8785	99.953	1.6956	99.742
51.3	1.9	51.0599	99.532	1.92378	101.251
Mean	-	-	99.86025	-	100.0936
± S.D.	-	-	0.323696	-	0.823829
%R.S.D	-	-	0. 324	-	0. 823

Table 2: Results for the determination of the studied drugs in their laboratory prepared mixtures by Method I.

Method II: For validation of the PCR, PLS and CLS models, the concentrations of MEB and CHL in the validation set were predicted by using the proposed PCR, PLS and CLS models. To evaluate the prediction performance of the proposed chemometric models, a set of 9 synthetic validation mixtures were prepared as shown in table 3, and submitted for prediction by each of the calibration models.

Concentration (µg/mL)		%Recovery MEB			%Recovery CHL		
MEB	CHL	CLS	PCR	PLS	CLS	PCR	PLS
32.4	1.2	99.42	98.60	98.52	102.58	101.22	101.57
32.4	1.4	99.41	98.64	98.55	101.30	99.85	99.77
37.8	1.2	100.47	100.0	100.10	102.66	102.12	101.98
37.8	1.4	100.74	100.23	100.27	101.04	100.16	99.84
37.8	1.6	101.20	100.36	100.17	102.02	100.57	101.12
43.2	1.6	100.81	100.14	99.97	102.58	101.58	102.61
48.6	1.6	100.18	99.73	99.73	102.46	101.92	102.26
48.6	1.8	101.18	100.61	100.48	100.32	99.49	100.33
48.6	2.0	100.68	100.07	99.85	101.44	100.36	101.18
Mean	-	100.45	99.82	99.74	101.82	100.81	101.18
± S.D.	-	0.668	0.722	0.717	0.835	0.939	1.032
%R.S.D.	-	0.67	0.72	0.72	0.82	0.93	1.02

Table 3: Results obtained for determination of MEB and CHL in synthetic mixtures using CLS, PCR, and PLS techniques by Method II.

Sample solution preparation

For method I and II, twenty Coloverin A tablets were accurately weighted, crushed and mixed well. Accurate weight of the powder tablets equivalent to 50 mg MEB and 1.85 mg CHL was transferred into a 50 mL volumetric flask to which about 30 mL methanol were then added. The solution was sonicated for 15 min with intermediate shaking. Finally, the volume was completed to the mark with methanol. The solution was filtered discarding first 10 mL and the filtrate was used as the sample solution.

Results and Discussions

Method development

Method I: The zero order spectra of MEB and CHL in methanol showed severe overlap that prevents the use of direct spectrophotometry for their analysis without preliminary separation (Figure 3). Derivative spectrophotometry is a well-established technique for the assay of drugs in mixtures and in pharmaceutical dosage forms enhancing the resolution of overlapping bands.

It can be applied for the determination of a drug in the presence of another by selecting a wavelength where contribution of one compound is almost zero while the compound to be determined has a reasonable value, so it has been used in the determination of many drugs [46]. By using the trough amplitudes of second derivative spectrum of MEB at 234.8 nm where CHL showed zero crossing ,and the trough amplitudes of first derivative of CHL at 280.4 nm, where MEB showed zero crossing (Figure 4) able to resolve such overlapping between MEB and CHL.
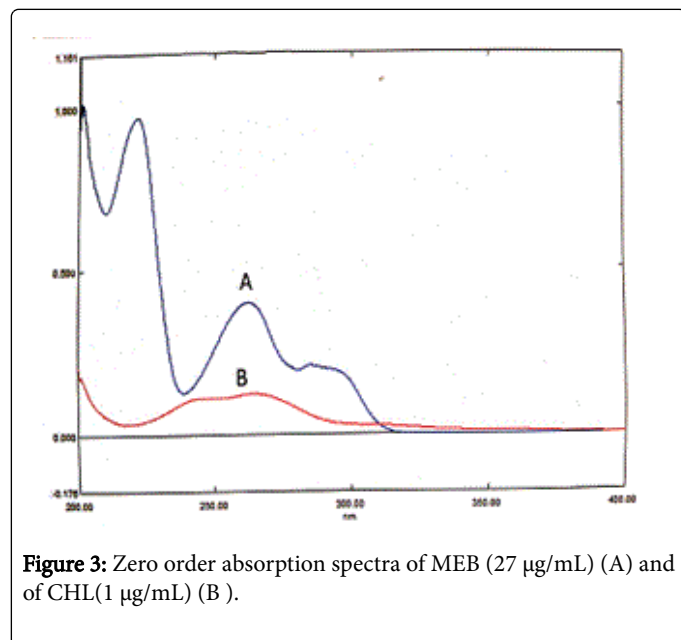

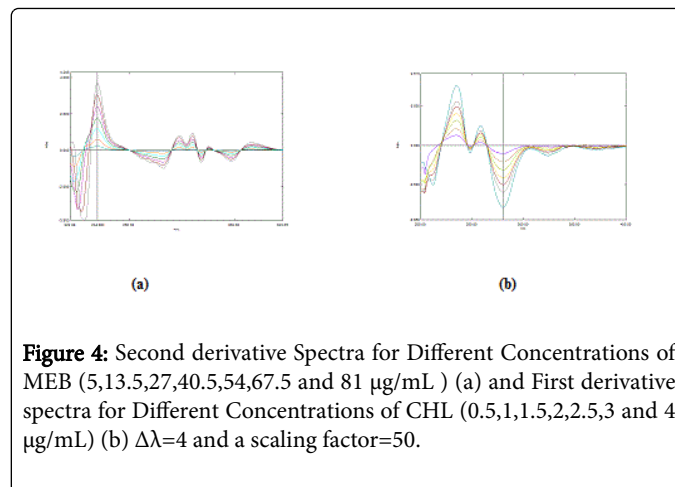**Figure 3:** Zero order absorption spectra of MEB (27 µg/mL) (A) and of CHL(1 µg/mL) (B).

Figure 4: Second derivative Spectra for Different Concentrations of MEB (5,13.5,27,40.5,54,67.5 and 81 µg/mL) (a) and First derivative spectra for Different Concentrations of CHL (0.5,1,1.5,2,2.5,3 and 4 µg/mL) (b) Δλ=4 and a scaling factor=50.

The zero crossing wavelengths at 234.8 nm for CHL and 280.4 nm for MEB were selected since reproducible readings were obtained at these wavelengths. A δλ of 4 and scaling factor of 50 were used for determination of MEB and CHL.

Method II: Three chemometric methods are applied CLS, PCR and PLS for simultaneous determination of the two drugs. The quality of multi component analysis depends on the wavelength range and the spectral mode used [47]. Twelve different concentration mixtures were used as the calibration samples to construct the models, the spectra of these mixtures were collected and examined, the regions from 200-260 nm and above 310 nm were rejected, however, the wavelength range 260-310 nm in the intervals with λ=0.2 nm was chosen as it provided the greatest amount of information about the mixture components. The ratio of the two drugs in Coloverin A tablets was taken in to consideration during the construction of both training and validation sets. CLS model was constructed with non-zero intercept. The non-zero intercept allows an additional degree of freedom when k matrix is calculated. This provides an additional opportunity to adjust the effects of the extraneous substances [48].

The CLS method requires all components in the calibration samples to be known. For the PCR and PLS techniques, selection of the optimum number of factors was a very important step before constructing the models. If the number of factors retained was more than the number required, more noise would be added to the data.

On the other hand, if the number retained was less than the number required, meaningful data that could be necessary for the calibration might be ignored. Visual inspection could be used for determining the optimum number of factors. Three factors were found suitable for both PCR and PLS methods and the plot of number of factors against RMSECV as presented in Figures (5) and (6).
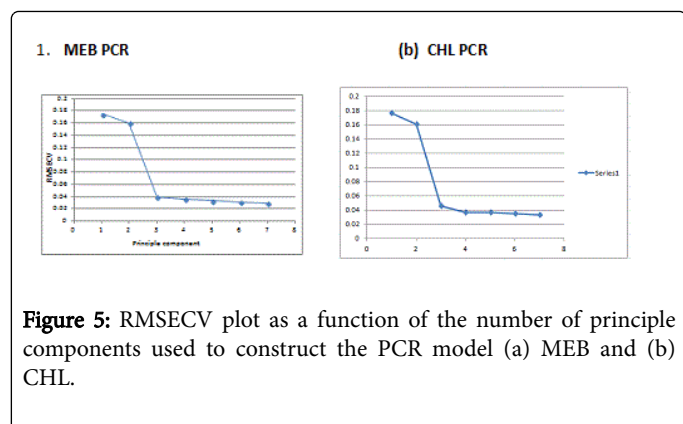
Figure 5: RMSECV plot as a function of the number of principle components used to construct the PCR model (a) MEB and (b) CHL.
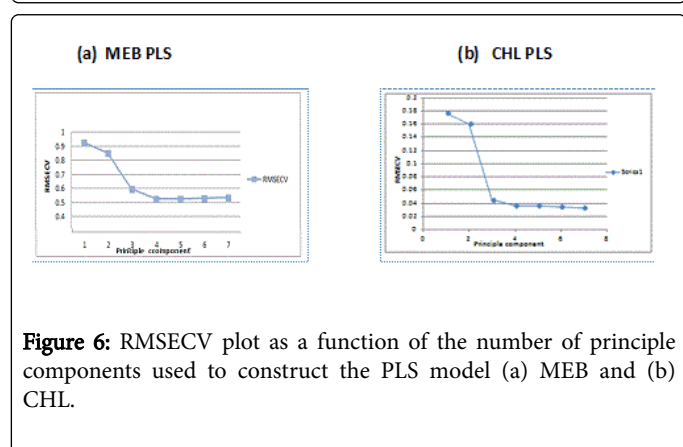

Figure 6: RMSECV plot as a function of the number of principle components used to construct the PLS model (a) MEB and (b) CHL.

Item	MEB	CHL
Derivative	2ndD	1stD
Wavelength of detection	234.8 nm	280.4 nm
Linearity range	5-81 µg/mL	0.5-4 µg/mL
Regression equation	Y=0.0451x-0.0258	Y=0.1981 × +0.0031
Regression coefficient (r2)	0.9997	0.9999
S.D.of slope (Sa)	0.01733	0.001749
S.D.of intercept (Sb)	0.000356	0.00074
C.I. for the slope	0.0451 ± 0.0009	0.1981 ± 0.001
C.I. for the intercept	-0.0258 ± 0.044	0.0031 ± 0.004
LOQ	0.388 µg/mL	0.150 µg/mL
LOD	0.128 µg/mL	0.05 µg/mL
Recovery for standard added	100.13 ± 0.935	100.65 ± 1.257
Recovery for drug in laboratory prepared mixtures	99.86 ± 0.323	100.093 ± 0.823
Accuracy	100.79 ± 0.64	100.24 ± 1.11
Intraday precision (%R.S.D, n=9)	0.21-0.93	0.21-0.81
Interday Precision (%R.S.D, n=9)	0.56-1.12	0.57-1.22

Table 4: Results obtained by for the determination of MEB and CHL Method I. Sa: standard deviation of intercept, Sb: standard deviation of slope and C.L: confidence limits.

Validation of Method I and Method II

The proposed methods were validated following the ICH guidelines [49].

Linearity: The linearity of the derivative spectrophotometric methods was evaluated by analyzing a series of different concentrations of each drug. In this study, seven concentrations for MEB and CHL, ranging from 5-81 µg/mL and 0.5-4 µg/mL, respectively in methanol were prepared.

Second derivative Spectra for MEB and first derivative spectra for CHL using the peak amplitude at 234.8 nm for MEB and the peak amplitude at 280.4 nm for CHL using Δλ=4 and a scaling factor=50, good linearity of the calibration curve was verified by the high correlation coefficient(r) of the regression equation. The analytical data of the calibration curve including standard deviations for the slope and intercept (Sb, Sa) are summarized in table 4.

Accuracy

For method I: The satisfactory recovery percentage results for the assay of MEB with CHL in their laboratory prepared mixtures at different concentration levels within the linear range of each compound (99.86 ± 0.323 for MEB and 100.093 ± 0.823 for CHL),indicate the accuracy of the method as shown in table (2). Also, the standard addition method was applied for the determination of MEB and CHL in Coloverin A tablets revealed good % recovery of added standard of MEB (100.13 ± 0.935) and CHL (100.65 ± 1.257) as shown in table (5) indicating the accuracy of the method in the dosage form by proposed derivative method.

Amount of tablet µg/mL		Amount of Added µg/mL		Tablet MEB		Tablet CHL		Added MEB		Added CHL	
MEB	CHL	MEB	CHL	Tablet Found µg/mL	% Recovery	Tablet found µg/mL	% Recovery	Added found µg/mL	% Recovery	Added found µg/mL	% Recovery
13.5	0.5	62.1	2.3	13.69	101.41	0.501	100.20	62.67	100.9	2.304	100.1
16.2	0.6	59.4	2.2	16.12	99.51	0.597	99.50	59.68	100.4	2.203	100.1
21.6	0.8	51.3	1.9	22.32	103.3	0.807	100.88	50.83	99.1	1.930	101.6

27	1.0	43.2	1.6	26.82	99.33	0.987	98.70	42.95	99.4	1.609	100.6
37.8	1.4	29.7	1.1	37.82	100.05	1.389	99.21	29.38	98.9	1.131	102.8
43.2	1.6	24.3	0.9	43.39	100.44	1.588	99.25	24.66	101.5	0.899	99.9
48.6	1.8	16.2	0.6	48.69	100.19	1.808	100.44	16.33	100. 8	0.592	98.7
51.3	1.9	13.5	0.5	51.23	99.86	1.909	100.47	13.50	100	0.507	101.4
Mean	-	-	-	-	100.515	-	99.831	-	100.13	-	100.65
± S.D.	-	-	-	-	1.294	-	0.768	-	0.935	-	1.257
%R.S.D.	-	-	-	-	1.29	-	0.77	-	0.93	-	1.25

Table 5: Results for the determination of MEB and CHL by standard addition method in drug formulation (Coloverin A tablets) by Method I.

For method II: The accuracy was assessed by applying the proposed chemometric methods obtained for the simultaneous determination of MEB and CHL as binary mixtures in pure form in concentration ranges of (32.4-48.6 µg/mL) for MEB and (1.2-2 µg/mL) for CHL. The mean percentage recoveries were found (100.45 ± 0.668, 99.82 ± 0.722 and 99.74 ± 0.717) for MEB and (101.82 ± 0.835, 100.81 ± 0.939 and 101.18 ± 1.032) for CHL as shown in table (3). Also accuracy of the drug product was confirmed by recovery studies from tablets at different levels of standard additions, the mean percentage recoveries of added were found (101.04 ± 1.62, 100.28 ± 1.61 and 100.08 ± 1.54) for MEB and (100.94 ± 1.35, 99.46 ± 0.96 and 100.98 ± 1.32) for CHL as shown in table (6).

Amount of tablet µg/mL		Amount of Added µg/mL		Tablet						Added					
				MEB %Recovery			CHL %Recovery			MEB %Recovery			CHL %Recovery		
MEB	CHL	MEB	CHL	CLS	PCR	PLS	CLS	PCR	PLS	CLS	PCR	PLS	CLS	PCR	PLS
18.9	0.7	27	1	104.71	104.36	104.51	103.66	103.98	102.80	99.34	99.02	99.07	99.26	99.41	99.05
27	1	24.3	0.9	101.86	101.53	101.74	102.63	102.43	100.82	100.90	100.72	100.69	101.45	101.20	101.43
32.4	1.2	21.6	0.8	103.60	103.16	103.27	103.50	103.77	102.87	99.61	98.28	98.02	102.02	98.39	100.26
37.8	1.4	18.9	0.7	102.15	101.72	101.84	100.34	100.61	99.72	101.18	99.88	99.50	102.69	98.82	101.63
40.5	1.5	16.2	0.6	102.84	102.56	102.79	98.63	99.01	97.26	103.89	102.87	102.42	100.63	99.33	102.93
43.2	1.6	13.5	0.5	102.3	101.81	101.86	95.22	95.54	95.23	101.34	100.90	100.78	99.61	99.60	100.58
Mean				102.91	102.52	102.67	100.66	100.89	99.78	101.04	100.28	100.08	100.94	99.46	100.98
± S.D.				1.07	1.08	1.09	3.30	3.23	3.05	1.622	1.61	1.54	1.35	0.96	1.32
%R.S.D.				1.03	1.05	1.06	3.27	3.20	3.05	1.60	1.60	1.53	1.33	0.96	1.30

Table 6: Results obtained for determination of MEB and CHL in Coloverin A tablets applying standard addition technique using CLS, PCR, and PLS techniques by Method II.

Precision: For method I and II Repeatability (intraday) and intermediate precision (interday) were assessed using three concentrations and three replicates of each concentration over 3 consecutive days. The results obtained including the mean of the recovery and standard deviation are displayed in tables (4, 7 and 8). The low RSD (< 1) indicated the good precision, reasonable repeatability and reflects the ruggedness of the proposed method.

	MEB			CHL		
Conc.	43.2 µg/mL	54 µg/mL	64.8 µg/mL	1.6 µg/mL	2 µg/mL	2.4 µg/mL
Mean	43.26	54.38	65.11	1.61	2.01	2.39
%Recovery	100.14	100.7	100.48	100.62	100.5	99.58

± S.D.	0.24	0.51	0.73	0.02	0.02	0.01
%R.S.D.	0.56	0.94	1.12	0.94	1.22	0.57

Table 7: Results for determination of inter-day assay for MEB and CHL in bulk powder by Method I.

Item	MEB			CHL		
	CLS	PCR	PLS	CLS	PCR	PLS
Intraday (%R.S.D.) For binary mixture	(0.16-0.59)	(0.17-0.60)	(0.16-0.60)	(0.09-0.84)	0.25-1.02)	(0.26-0.85)
Interday (%R.S.D.) For binary mixture	(0.43-0.70)	(0.40-0.68)	(0.40-0.69)	(0.73-1.29)	(1.11-1.28)	(1.17-1.86)

Table 8: Intraday and Interday results for determination of MEB and CHL by Method II.

Selectivity: It was found that MEB and CHL can be determined simultaneously either in their laboratory prepared mixtures or in their co formulated tablets as shown in table (2) with adequate accuracy and precision. The UV scans of the dosage form overlay those of the laboratory prepared mixtures indicating absence of interference due to excipients in the dosage form. The results displayed in tables (2 and 3) demonstrate good recoveries and absence of interference from other materials in pharmaceutical dosage forms (Coloverin A tablets) and in laboratory prepared mixtures, confirm the selectivity of the methods.

Limit of detection and limit of quantification: According to the ICH recommendations, [49] the approach based on the SD of the response and the slope was used for determining the limit of detection and limit of quantification, using the equations:

LOD=3.3xSD / slope, LOQ=10xSD/slope

Conclusion

The proposed methods have the advantages of simplicity, precision, accuracy and convenience for the quantization of MEB and CHL in their combined dosage form. The methods were validated for the drug substances and drug product by means of the analysis of typical performance characteristics such as robustness, selectivity, linearity, accuracy, precision, and system suitability, according to ICH guidelines. Results showed that the developed methods can be applied to a routine analysis, quality control of the cited drugs in ordinary laboratories.

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