Hindawi Publishing Corporation
Journal of Chemistry
Volume 2015, Article ID 293719, 9 pages
http://dx.doi.org/10.1155/2015/293719

Research Article

Simultaneous HPLC Determination of Chlordiazepoxide and Mebeverine HCl in the Presence of Their Degradation Products and Impurities

Rania N. El-Shaheny and Fathalla F. Belal

Analytical Chemistry Department, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

Correspondence should be addressed to Rania N. El-Shaheny; rania_n2010@yahoo.com

Received 8 December 2014; Revised 10 February 2015; Accepted 10 February 2015

Academic Editor: Victor David

A simple, rapid, and sensitive RP-HPLC method was developed and validated for the simultaneous determination of chlordiazepoxide (CDO) and mebeverine HCl (MBV) in the presence of CDO impurity (2-amino-5-chlorobenzophenone, ACB) and MBV degradation product (veratric acid, VER). Separation was achieved within 9 min on a BDS Hypersil phenyl column (4.5 mm × 250 mm, 5 μm particle size) using a mobile phase consisting of acetonitrile: 0.1 M potassium dihydrogen phosphate: triethylamine (35 : 65 : 0.2, v/v/v) in an isocratic mode at a flow rate of 1 mL/min. The pH of the mobile phase was adjusted to 4.5 with orthophosphoric acid and UV detection was set at 260 nm. A complete validation procedure was conducted. The proposed method exhibited excellent linearity over the concentration ranges of 1.0–100.0, 10.0–200.0, 2.0–40.0, and 2.0–40.0 μg/mL for CDO, MBV, VER, and ACB, respectively. The proposed method was applied for the simultaneous determination of CDO and MBV in their coformulated tablets with mean percentage recoveries of 99.75 ± 0.62 and 98.61 ± 0.38, respectively. The results of the proposed method were favorably compared with those of a comparison HPLC method using Student *t*-test and the variance ratio *F*-test. The chemical structure of MBV degradation product was ascertained by mass spectrometry and IR studies.

1. Introduction

Chlordiazepoxide (CDO, Figure 1(a)), 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine 4-oxide, is a benzodiazepine with general properties similar to those of diazepam. It is used for the short-term treatment of anxiety disorders and insomnia. CDO is also used in muscle spasm and in alcohol withdrawal syndrome [1]. Mebeverine hydrochloride (MBV, Figure 1(b)), 4-[ethyl(4-methoxy-α-methylphenethyl)amino]butyl veratrate hydrochloride, is an antispasmodic with a direct action on the smooth muscles of the gastrointestinal tract. It is used in conditions such as irritable bowel syndrome [1]. The combined therapy of MBV, as an antispasmodic agent, with the anxiolytic drug CDO provides effective relief of spastic colon and associated symptoms of anxiety and tension in the gastrointestinal tract.

CDO is the subject of a monograph in the British Pharmacopoeia (BP) [2] and the United States Pharmacopoeia (USP) [3]. The BP [2] recommends a potentiometric non-aqueous titration method with 0.1 M perchloric acid as a titrant for its determination in pure form and a spectrophotometric method for its determination in capsules. The USP [3] describes HPLC methods for its determination in pure form and single or coformulated tablets with either clidinium bromide or amitriptyline HCl. Literature survey revealed many analytical methods for the determination of CDO either alone or in combination with other drugs. Some examples of these methods include ion-selective electrode [4], spectrophotometry [5–8], and HPLC [9–11]. Some methods were also applied to separate CDO from its photodegradation products [12] or impurities [13].

MBV is also an official drug in the BP [2] that recommends a potentiometric non-aqueous titration method with

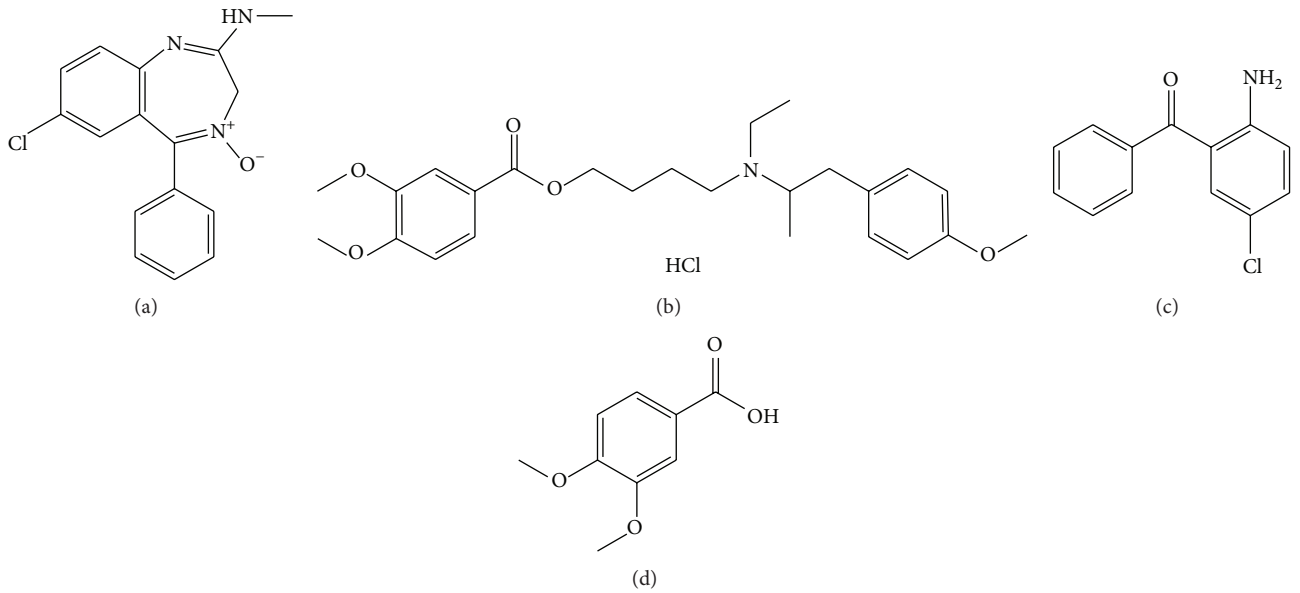

Figure 1: Structural formulas of (a) chlordiazepoxide, (b) mebeverine HCl, (c) 2-amino-5-chlorobenzophenone, and (d) veratric acid.

0.1 M perchloric acid and a spectrophotometric method for its determination in pure form and tablets, respectively. Many analytical methods were applied for determination of MBV such as spectrophotometry [14–17], TLC [15], HPLC [14, 15, 18, 19], and spectrofluorimetry [20].

Reviewing the scientific literature revealed an HPLC method [21] and two spectrophotometric methods based on the simultaneous equations and absorbance ratio method [22] for the simultaneous estimation of CDO and MBV in their coformulated tablets. These methods are not stability-indicating; moreover, the pharmaceutical ratio of the two drugs (1 CDO : 27 MBV) was not achieved in the two spectrophotometric methods [22] which are linear over the ranges of 1.0–10.0 and 2.0–12.0 μg/mL, for CDO and MBV, respectively, so they are not practically applicable to coformulated dosage forms. Only one HPLC method [23] was published as a stability-indicating assay for the two drugs' combination. Nevertheless, this method suffers from some drawbacks such as poor sensitivity, narrow linearity range, and complicated gradient elution program. Hence, our main target was to develop a simple, sensitive, and rapid isocratic stability-indicating HPLC method for the simultaneous determination of the two drugs in their combined tablets in the presence of their degradation products or synthesis impurities.

The BP [2] and the USP [3] define 2-amino-5-chlorobenzophenone (ACB, Figure 1(c)) as an impurity in CDO pure and dosage forms. ACB is the starting material for synthesis of many diazepams including CDO [24]. On the other hand MBV, being an ester-containing drug, is easily susceptible to hydrolytic degradation giving the corresponding acid degradation product, veratric acid (3,4-dimethoxy benzoic acid, VER, Figure 1(d)) [25]. Moreover, the BP [2] defines VER as a related substance in MBV pure form and tablets.

In this study, we developed a stability-indicating HPLC method for the analysis of CDO and MBV in combination and in the presence of MBV degradation product (VER) and CDO synthetic precursor and main impurity (ACB). The identification of the degradation product of MBV was ascertained by mass spectrometry and IR studies. The developed method was validated according to ICH Guidelines [26]. The proposed procedure is suitable for the quality control analysis and purity assessment of dosage forms containing the two studied compounds.

2. Experimental

2.1. Instruments. Separation was performed using a Shimadzu HPLC system (Shimadzu Corporation, Japan) consisting of LC-20AD liquid chromatograph equipped with a Rheodyne injector valve and a 20 μL sample loop, a SPD 20A UV-Vis detector, DGU-20A5 online solvent degasser, and CBM-20A communication bus module. A Consort P-901 pH-meter (Turnhout, Belgium) was used for pH adjustment.

2.2. Materials. Gift samples of chlordiazepoxide and mebeverine HCl were kindly provided by EVA Pharma Co. (Cairo, Egypt). Their purities are 100.10 and 100.12%, respectively as determined by the reference method [21]. 2-Amino-5-chlorobenzophenone was purchased from Sigma Aldrich Co. (Steinheim am Albuch, Germany). Coloverin A film coated tablets labeled to contain 5 mg CDO + 135 mg MBV/tablet (batch number 120960A), product of Chemipharm Pharmaceutical Industries S.A.E., 6th of October city, Egypt, were purchased from a local pharmacy.

2.3. Reagents. All solvents used were of HPLC grade and all chemicals were of analytical reagent grade. High purity water,

obtained by filtration of distilled water through a 0.45 μm membrane filter, was used throughout the study. Acetonitrile and methanol (HPLC grade) were obtained from Sigma Aldrich Co. (Steinheim am Albuch, Germany). Orthophosphoric acid (85%, w/v) and triethylamine (TEA) were obtained from Riedel-de Häen (Seelze, Germany). Potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, ethanol, methylene chloride, and diethyl ether were obtained from ADWIC Co. (Cairo, Egypt).

2.4. Preparation and Isolation of Veratric Acid. Veratric acid was prepared in our laboratory according to the method of Naguib and Abdelkawy [27]. 0.5 gm of MBV was dissolved in 25 mL of 1 M NaOH methanolic solution. The solution was heated at 45°C for 12 h till complete degradation of MBV as confirmed by the disappearance of MBV spot upon applying qualitative TLC on silica gel F_{254} plates using absolute ethanol: methylene chloride: TEA (7 : 3 : 0.2 v/v/v) as a mobile phase [27]. The solution was evaporated to a residue. The residue was dissolved in 20 mL distilled water and extracted with diethyl ether (3 × 15 mL). The aqueous layer containing VER was separated and acidified with 5 N HCl till acidic to litmus paper, where a heavy white precipitate of VER was produced which was then filtered and washed with distilled water (3 × 15 mL). The white needles of VER were dried in an oven at 90°C for 1 h. The identity of VER was confirmed by mass spectrometry and IR studies.

2.5. Chromatographic Conditions. Separation was performed on a BDS Hypersil phenyl column (4.5 mm × 250 mm, 5 μm particle size) from Thermo Electron Corporation (Runcorn, UK), using a mobile phase consisted of a ternary mixture of acetonitrile: 0.1 M potassium dihydrogen phosphate: TEA (35 : 65 : 0.2, v/v/v). The final pH of the mixed mobile phase was adjusted to 4.5 with orthophosphoric acid and it was delivered at a flow rate of 1 mL/min. The detector wavelength was set at 260 nm.

2.6. Standard Stock Solutions. Standard stock solutions of CDO (400.0 μg/mL), MBV (400.0 μg/mL), and VER (200.0 μg/mL) were prepared individually in methanol. ACB standard stock solution (200.0 μg/mL) was prepared in acetonitrile. The standard stock solutions were found to be stable for 2 weeks when kept in a refrigerator at 4°C.

2.7. Recommended Procedures

2.7.1. Construction of Calibration Graphs. Seven-point calibration graphs were constructed by quantitatively transferring increasing volumes of the standard stock solutions of CDO (25 μL-2.5 mL), MBV (0.25–2.5 mL), ACB (0.1–2.0 mL), and VER (0.1–2.0 mL) to four sets of 10 mL volumetric flasks. The solutions were made up to the volume with the mobile phase and mixed well to obtain final concentration ranges of 1.0–100.0, 10.0–200.0, 2.0–40.0, and 2.0–40.0 μg/mL for CDO, MBV, ACB, and VER, respectively. Twenty μL aliquots were injected in triplicate and eluted under the optimum chromatographic conditions. Average peak areas of each compound were plotted versus the corresponding concentration (μg/mL) to obtain the calibration graphs and the corresponding regression equations were derived.

2.7.2. Analysis of Laboratory-Prepared Mixtures of CDO/MBV. For the analysis of binary mixtures of the two studied drugs in their pharmaceutical ratio (1 CDO: 27 MBV), a mixed standard stock solution containing both CDO and MBV (100.0 and 2700.0 μg/mL, resp.) was prepared in methanol. From this solution aliquots of 0.3, 0.5, and 0.7 mL were transferred into a series of 10 mL volumetric flasks. The solutions were made up to volume with the mobile phase to obtain mixtures with final concentrations of 3.0 CDO + 81.0 MBV, 5.0 CDO + 135.0 MBV, and 7.0 CDO + 189.0 MBV, respectively. The solutions were mixed well and 20 μL aliquots were injected in triplicate and eluted under the optimum chromatographic conditions. The average percentage recovery of each compound was determined from the corresponding regression equations.

2.7.3. Analysis of Laboratory-Prepared Mixtures of CDO/ACB and MBV/VER. Aliquots of 2.5 mL of the standard solution of CDO were transferred into a series of 10 mL volumetric flasks together with increasing volumes (0.1, 0.2, 0.4, and 0.5 mL) of its impurity (ACB). Similarly, aliquots of 5.0 mL of MBV standard solution were transferred into a series of 10 mL volumetric flasks together with increasing volumes (0.1, 0.2, 0.4, and 0.5 mL) of its degradation product and impurity (VER). The volumes were completed to the mark with the mobile phase. Solutions were mixed well and 20 μL were injected (triplicate). Samples were eluted under the optimum chromatographic conditions. The average percentage recoveries of CDO and MBV were calculated from the corresponding regression equations.

2.7.4. Analysis of Tablets. Ten tablets were accurately weighed and finely powdered. An amount of the powder equivalent to 5 mg CDO + 135 mg MBV was weighed and transferred into 50 mL volumetric flask. About 40 mL of methanol was added and the solution was sonicated for about 30 min. The volume was made up to the mark with the same solvent; solution was mixed well and filtered through cellulose acetate-syringe filtration disks. The obtained tablet extract contains CDO and MBV in the concentrations of 100.0 and 2700.0 μg/mL, respectively. Different volumes of this solution (0.3, 0.5, and 0.7 mL) were transferred into a series of 10 mL volumetric flasks; the volumes were made up to the mark with the mobile phase and mixed well. 20 μL aliquots were injected in triplicate and eluted under the optimum chromatographic conditions. The average percentage recoveries of CDO and MBV were calculated from the previously derived regression equations.

3. Results and Discussion

A fast, reliable, and selective HPLC method was developed and validated for the simultaneous analysis of CDO and MBV in the presence of their impurities and degradation

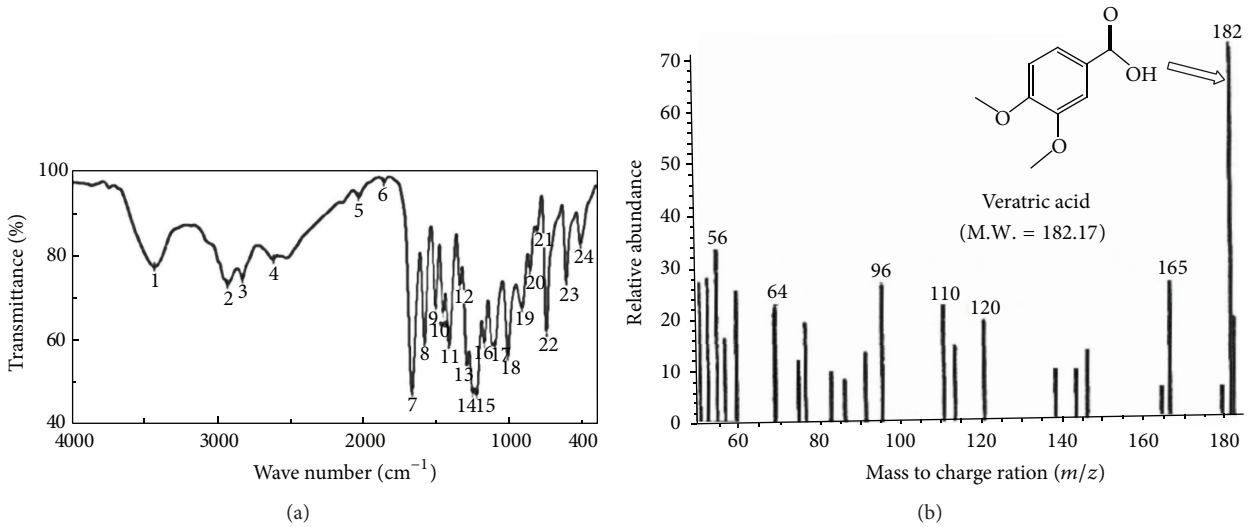

Figure 2: (a) IR spectrum and (b) mass spectrum of veratric acid.

products. The degradation product of MBV was identified by mass spectrometry exhibiting a molecular ion peak at mass to charge ration (m/z) of 182 which is consistent with 3,4-dimethoxybenzoic acid (veratric acid, VER) formed as a result of the hydrolysis of the ester linkage of MBV. The IR spectrum of the degradation product showed an intense band at 1705 cm^{-1} corresponding to the carbonyl stretch C=O of a carboxylic acid and a strong broad band centered around 3000 cm^{-1} corresponding to the carboxylic acid O–H stretch [28], thus confirming the structure of VER. Figure 2 illustrates the mass and IR spectra of VER.

Hence, the separation of the two drugs and their corresponding degradation products and impurities was optimized as illustrated below.

3.1. Optimization of Chromatographic Conditions. Several mobile phases were tried using various portions of organic modifier and buffer of different molarities at different pH values. The ratio of acetonitrile has a marked effect on the retention of the four compounds. A ratio of 35% (v/v) acetonitrile, was selected as the optimum yielding well separated peaks for the four compounds with sufficient sensitivity and suitable peak symmetry (peaks tailing factor <1.6) in a short run time (<9 min). At a ratio of 40% (v/v) acetonitrile, the chromatographic peak of VER strongly overlapped with that of CDO, and that of MBV overlapped with that of ACB. On the other hand, decreasing the ratio of acetonitrile (≤30%, v/v) led to longer retention times, especially for MBV and ACB.

Phosphate buffer of different ionic strengths (0.025–0.1 M) was investigated and 0.1 M was chosen as the optimum concentration allowing the simultaneous determination of the four compounds within a reasonable run time. At lower ionic strengths, the retention of MBV and ACB increased with asymmetric distorted peaks which are completely overlapped when using buffers at concentrations of 0.025 to 0.05 M, with no significant effect on CDO and VER peaks.

Increasing the pH of the mobile phase resulted in an increase of the retention of CDO, MBV, and ACB, without a significant change in the retention of VER. At pH 4.5, optimum resolution with reasonable retention times was achieved. At higher pH values, unacceptable long retention times of MBV and ACB were observed, where, at lower pH values, the resolution between VER/CDO and MBV/ACB pairs was adversely affected.

TEA was added to the mobile phase in order to improve peak symmetry and sharpness. It prevents the interaction of the basic compounds (MBV and ACB) with the phenyl-bonded stationary phase, where TEA associates with the silanol sites blocking ion-exchange processes. When using mobile phase deprived of this ion suppressing agent, the retention times of both MBV and ACB increased, probably due to interaction of these basic drugs with the silanol groups of silica, and they were coeluted at 12 min. The concentration of TEA was kept at 0.2 mL for achieving best peak symmetry, where, at lower concentrations, broadening as well as distortion of the peaks was observed.

For selection of the optimum detection wavelength, the absorption spectra of the four analytes were scanned in the mobile phase. MBV exhibited two absorption maxima at 260 and 290 nm and VER at 256 and 288 nm. CDO shows maximum absorption at 250 nm, while ACB has highest absorption intensity at 260 nm. For the simultaneous detection and determination of the four components with high sensitivity, the UV detection was carried out at 260 nm since all compounds show appreciable absorption at this wavelength.

A study of the influence of the flow rate of the mobile phase on the separation process was also carried out over the range of 0.8–1.2 mL/min. At a flow rate of 0.8 mL/min, the

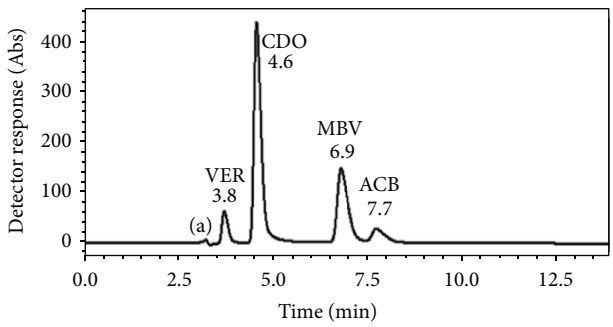

Figure 3: Representative chromatogram showing good separation of CDO (50.0 μg/mL), MBV (100.0 μg/mL), veratric acid (10.0 μg/mL), and ACB (10.0 μg/mL), where (a) is the solvent front.

analysis time increased and broad peaks were obtained, while, at a flow rate of 1.2 mL/min, the resolution of VER/CDO and MBV/ACB pairs was adversely affected. A flow rate of 1.0 mL/min was chosen as the optimum flow rate allowing good resolution of the four peaks of the studied compounds within a reasonable run time (<9 min).

After this thorough experimental study good separation between the two drugs and their degradation products and impurities was achieved. Figure 3 shows a typical chromatogram for the separation of the four compounds at retention times (t_R) of 3.8, 4.6, 6.9, and 7.7 min for VER, CDO, MBV, and ACB, respectively.

3.2. Method Validation. Method validation was performed according to ICH guidelines [26] to evaluate the suitability of the method for separation and quantitative determination of CDO, MBV, and their degradation products. A summary of the validation results is illustrated below.

3.2.1. Linearity and Range. The linearity of the proposed method was evaluated by processing 7-point calibration graph for each compound. The peak area of each drug was plotted versus its nominal concentration (μg/mL), and a linear regression analysis was conducted to demonstrate the linearity of the method [29]. The small values of the standard deviations of the residuals ($S_{y/x}$), slope (S_b), and intercept (S_a) and the % relative error indicate good linearity of the proposed method (Table 1).

3.2.2. Limit of Quantification (LOQ) and Limit of Detection (LOD). Both LOQ and LOD were calculated according to ICH guidelines using the following equations [26]:

$$\text{LOQ} = \frac{10S_a}{b}, \quad (1)$$

$$\text{LOD} = \frac{3.3S_a}{b}, \quad (2)$$

where S_a is the standard deviation of the intercept of regression line and b is the slope of the regression line. The results are summarized in Table 1.

3.2.3. Accuracy. The accuracy was studied by applying the proposed method for the determination of pure samples of CDO and MBV over the working concentration ranges. The mean percentage recovery ± SD was calculated and the results are presented in Table 2. The obtained results were statistically compared with those obtained by a comparison HPLC method [21] using Student t-test and the variance ration F-test revealing no significant difference regarding the accuracy and precision, respectively [29].

3.2.4. Precision. The intraday precision was assessed from the results of the analyses of quality control samples of three different concentrations at three successive times in the same day. The interday precision was also determined from three quality control samples analyzed on 3 consecutive days [26]. The results of intraday and interday precision illustrated in Table 3 indicate the high precision of the proposed method.

3.2.5. Selectivity. The selectivity of the proposed method was proven by its ability to separate the two drugs from their degradation products and impurities. Different mixtures of CDO/MBV, CDO/ACB, and MBV/VER were prepared and analyzed according to the previously mentioned procedure. The recovery, % RSD, and % error values shown in Table 4 were satisfactory, thus proving the selectivity and accuracy of the proposed method and its ability to separate the drugs from each other and from degradation products and impurities. Moreover, no interference with the peaks of interest was observed from common tablet excipients.

3.2.6. System Suitability. Resolution (R_s), selectivity factor (α) (both were calculated between each two adjacent peaks), retention factor (k'), number of theoretical plates (N), and tailing factor (T) were calculated as *per* USP guidelines as criteria for system suitability test [3] (Table 5).

3.2.7. Robustness. The reliability of the proposed method with respect to deliberate variations in method parameters was studied. The influences of variation of pH of the mobile phase (4.5 ± 0.2), ratio of acetonitrile in the mobile phase (35 ± 1%, v/v), and molar strength of phosphate buffer (0.1 ± 0.005 M) were examined. These minor deliberate variations in the method parameters did not significantly affect the resolution or peak area of the four test compounds.

3.3. Application of the Proposed Method for Quality Control of Coformulated Tablets Containing CDO and MBV Mixture. The applicability of the validated HPLC method to the simultaneous determination of MBV and CDO was verified by the analysis of their coformulated Coloverin A tablets. The obtained results were statistically compared with those of the comparison method [21] by Student t-test and the variance ratio F-test revealing no significant differences [29]. The results in Table 2 demonstrate the quality of the analyzed pharmaceutical samples and the applicability of the method for quality control analysis. The obtained results were in accordance with the labeled claim amount. The results of the assay indicate that the method is selective for the

Table 1: Analytical performance data for determination of CDO, MBV, VER, and ACB by the proposed method.

Parameter	Result			
	CDO	MBV	VER	ACB
Concentration range (μg/mL)	1.0–100.0	10.0–200.0	2.0–40.0	2.0–40.0
Limit of detection (LOD) (μg/mL)	0.23	0.67	0.05	0.15
Limit of quantification (LOQ) (μg/mL)	0.71	2.04	0.15	0.45
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Slope	1.05×10^5	2.92×10^4	6.93×10^4	6.87×10^4
Intercept	2.15×10^4	1.08×10^4	-1.76×10^4	7.34×10^4
Standard deviation of the residuals ($S_{y/x}$)	1.50×10^4	9.98×10^3	3.81×10^3	8.85×10^3
Standard deviation of the intercept (S_a)	7.45×10^3	5.95×10^3	1.04×10^3	3.12×10^3
Standard deviation of the slope (S_b)	1.70×10^2	0.63×10^2	1.09×10^2	2.81×10^2
% RSD	0.91	0.99	0.94	0.94
% error (% RSD/$\sqrt{n}$)	0.34	0.37	0.35	0.38

Table 2: Application of the proposed and comparison methods for the determination of CDO and MBV in pure form and coformulated tablets.

Matrix	Proposed method				Comparison method [21]	
	CDO		MBV		CDO	MBV
	Conc. taken (μg/mL)	% found[a]	Conc. taken (μg/mL)	% found[a]	% found[a]	
Pure form	1.0	98.00	10.0	98.17	101.40	101.53
	2.5	98.60	20.0	98.25	98.30	98.16
	5.0	100.00	40.0	100.10	100.60	100.66
	15.0	100.06	60.0	100.10		
	25.0	99.52	80.0	100.39		
	50.0	100.55	100.0	100.46		
	100.0	99.89	200.0	99.83		
Mean ± SD		99.52 ± 0.90		99.61 ± 0.98	100.10 ± 1.61	100.12 ± 1.75
t-value		0.753 (2.306)[b]		0.597 (2.306)[b]		
F-value		3.187 (5.14)[b]		3.176 (5.14)[b]		
Coloverin A tablets (5 mg CDO + 135 mg MBV/tablet)	3.0	100.22	81.0	98.25	101.20	100.88
	5.0	99.98	135.0	99.01	98.58	98.95
	7.0	99.05	189.0	98.58	100.51	100.38
Mean ± SD		99.75 ± 0.62		98.61 ± 0.38	100.10 ± 1.36	100.07 ± 1.00
t-value		0.402 (2.776)[b]		2.354 (2.776)[b]		
F-value		4.829 (19.00)[b]		6.91 (19.00)[b]		

[a]Each result is the average of three separate determinations.
[b]Values between parentheses are the tabulated t- and F-values at $P = 0.05$ [29].

Table 3: Precision data for the determination of CDO and MBV by the proposed method.

Compound	Conc. (μg/mL)	Intraday precision		Interday precision	
		Mean % found ± SD	% RSD	Mean % found ± SD	% RSD
CDO	1.0	100.10 ± 0.91	0.91	99.45 ± 1.07	1.08
	50.0	100.77 ± 1.44	1.43	99.15 ± 0.95	0.95
	100.0	100.44 ± 0.55	0.55	100.50 ± 0.64	0.63
MBV	10.0	100.83 ± 0.63	0.62	99.09 ± 0.81	0.82
	100.0	100.41 ± 0.67	0.67	99.49 ± 1.33	1.33
	200.0	99.75 ± 0.53	0.53	100.42 ± 1.09	1.09

Table 4: Application of the proposed method for the determination of CDO, MBV, and their degradation products in binary mixtures.

Parameter	Conc. taken (μg/mL)		% found[a]	
	CDO	MBV	CDO	MBV
Binary mixture of CDO/MBV	3.0	81.0	99.82	99.87
	5.0	135.0	98.52	100.58
	7.0	189.0	98.65	99.08
Mean ± SD			98.99 ± 0.72	99.84 ± 0.75
% RSD			0.72	0.75
% error			0.41	0.43
	Conc. taken (μg/mL)		% found of CDO[a]	
	CDO	ACB		
Binary mixture of CDO/ACB	100.0	2.0	99.08	
	100.0	4.0	98.70	
	100.0	8.0	99.12	
	100.0	10.0	100.01	
Mean ± SD			99.22 ± 0.56	
% RSD			0.56	
% error			0.32	
	Conc. taken (μg/mL)		% found of MBV[a]	
	MBV	VER		
Binary mixture of MBV/VER	200.0	2.0	100.20	
	200.0	4.0	99.68	
	200.0	8.0	100.03	
	200.0	10.0	99.98	
Mean ± SD			99.97 ± 0.22	
% RSD			0.22	
% error			0.08	

[a]Each result is the average of three separate determinations.

Table 5: Final system suitability test parameters for the proposed method.

Compound	Number of theoretical plates (N)	Retention factor (k')	Tailing factor (T)
CDO	2778	0.42	1.44
MBV	2542	1.13	1.55
VER	2433	0.15	1.52
ACB	2326	1.40	1.48
Compounds[a]	Resolution (R_s)	Selectivity factor (α)	
CDO/VER	2.64	2.80	
CDO/MBV	5.04	2.69	
MBV/ACB	1.45	1.24	

[a]Resolution and selectivity factor were calculated for each two adjacent peaks.
Calculations were done according to USP guidelines [3].

assay of MBV and CDO without interference from common tablet excipients. A typical chromatogram obtained from the analysis of tablets is shown in Figure 4. As can be seen, no degradation of the sample was observed indicating the stability of tablets under storage conditions.

4. Conclusion

A fast, simple, and accurate stability-indicating HPLC method has been developed for the simultaneous determination of MBV and CDO and their impurities and degradation products, that are, VER and ACB, respectively. The method allows identification, quantitative analysis, and purity assessment to be performed simultaneously. The proposed method appears to be suitable for quality control laboratories, where economy and time-saving are essential.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

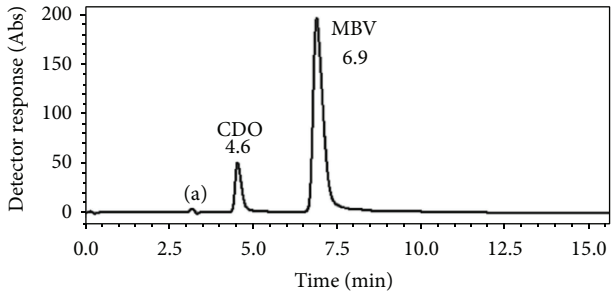

Figure 4: Representative chromatogram obtained for simultaneous determination of CDO (5.0 μg/mL) and MBV (135.0 μg/mL) in coformulated Coloverin A tablets, where (a) is the solvent front.

Authors' Contribution

Rania N. El-Shaheny proposed the method and carried out the experimental work and statistical analysis of the data. Fathalla F. Belal supervised the work. Both authors approved the final paper.

References

[1] S. C. Sweetman, *Martindale: The Complete Drug Reference*, The Pharmaceutical Press, London, UK, 36th edition, 2009.

[2] *The British Pharmacopoeia*, Her Majesty's Stationary Office, London, UK, 2012.

[3] *The United States Pharmacopoeia 35th and The National formulary 30th*, United Book Press, Rockville, Md, USA, 2012.

[4] I. P. Gorelov, S. S. Ryasenskii, S. V. Kartamyshev, and M. V. Fedorova, "A solid-state ionic-selective electrode with an ionic-electronic transducer for determining chlordiazepoxide," *Journal of Analytical Chemistry*, vol. 60, no. 1, pp. 65–69, 2005.

[5] M. R. Khoshayand, H. Abdollahi, A. Moeini, A. Shamsaie, A. Ghaffari, and S. Abbasian, "Simultaneous spectrophotometric determination of chlordiazepoxide and clidinium using multivariate calibration techniques," *Drug Testing and Analysis*, vol. 2, no. 9, pp. 430–435, 2010.

[6] M. I. Toral, P. Richter, N. Lara, P. Jaque, C. Soto, and M. Saavedra, "Simultaneous determination of chlordiazepoxide and clidinium bromide in pharmaceutical formulations by derivative spectrophotometry," *International Journal of Pharmaceutics*, vol. 189, no. 1, pp. 67–74, 1999.

[7] A. H. M. Sarrafi, Z. Khodakarami, and M. Karkeabadi, "Simultaneous spectrophotometric determination of amitriptyline hydrochloride and chlordiazepoxide in pharmaceutical tablets by multivariate calibration method," *E-Journal of Chemistry*, vol. 6, supplement 1, pp. S111–S116, 2009.

[8] S. Patel, N. J. Patel, and S. A. Patel, "Simultaneous spectrophotometric estimation of imipramine hydrochloride and chlordiazepoxide in tablets," *Indian Journal of Pharmaceutical Sciences*, vol. 71, no. 4, pp. 468–472, 2009.

[9] K. B. Borges, E. F. Freire, I. Martins, and M. E. P. B. de Siqueira, "Simultaneous determination of multibenzodiazepines by HPLC/UV: investigation of liquid-liquid and solid-phase extractions in human plasma," *Talanta*, vol. 78, no. 1, pp. 233–241, 2009.

[10] A. Pathak, P. Rai, and S. J. Rajput, "Stability-indicating HPLC method for simultaneous determination of clidinium bromide and chlordiazepoxide in combined dosage forms," *Journal of Chromatographic Science*, vol. 48, no. 3, pp. 235–239, 2010.

[11] S. Khodadoust and M. Ghaedi, "Optimization of dispersive liquid-liquid microextraction with central composite design for preconcentration of chlordiazepoxide drug and its determination by HPLC-UV," *Journal of Separation Science*, vol. 36, no. 11, pp. 1734–1742, 2013.

[12] V. Soentjens-Werts, J. G. Dubois, G. Atassi, and M. Hanocq, "Chlordiazepoxide photoisomerization kinetics into oxaziridine. A HPLC study," *Talanta*, vol. 42, no. 4, pp. 581–589, 1995.

[13] S. E. Roberts and M. F. Delaney, "Determination of chlordiazepoxide, its hydrochloride and related impurities in pharmaceutical formulations by reversed-phase high-performance liquid chromatography," *Journal of Chromatography*, vol. 283, pp. 265–272, 1984.

[14] E. A. Abdelaleem and N. S. Abdelwahab, "Validated chromatographic and spectrophotometric methods for analysis of some amoebicide drugs in their combined pharmaceutical preparation," *Pakistan Journal of Pharmaceutical Sciences*, vol. 26, no. 1, pp. 175–183, 2013.

[15] A. F. M. El Walily, A. El Gindy, and M. F. Bedair, "Application of first-derivative UV-spectrophotometry, TLC-densitometry and liquid chromatography for the simultaneous determination of mebeverine hydrochloride and sulpiride," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 21, no. 3, pp. 535–548, 1999.

[16] A. M. El-Didamony, "Spectrophotometric determination of benzydamine HCl, levamisole HCl and mebeverine HCl through ion-pair complex formation with methyl orange," *Spectrochimica Acta—Part A: Molecular and Biomolecular Spectroscopy*, vol. 69, no. 3, pp. 770–775, 2008.

[17] S. A. Shama and A. S. Amin, "Spectrophotometric microdetermination of nefopam, mebevrine and phenylpropanolamine hydrochloride in pharmaceutical formulations using alizarins," *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy*, vol. 60, no. 8-9, pp. 1769–1774, 2004.

[18] M. S. Arayne, N. Sultana, and F. A. Siddiqui, "A new RP-HPLC method for analysis of mebeverine hydrochloride in raw materials and tablets," *Pakistan Journal of Pharmaceutical Sciences*, vol. 18, no. 2, pp. 11–14, 2005.

[19] M. A. Radwan, H. H. Abdine, and H. Y. Aboul-Enein, "A validated chiral HPLC method for the determination of mebeverine HCl enantiomers in pharmaceutical dosage forms and spiked rat plasma," *Biomedical Chromatography*, vol. 20, no. 2, pp. 211–216, 2006.

[20] M. Walash, M. Sharaf El-Din, N. El-Enany, M. Eid, and Sh. Shalan, "First derivative synchronous fluorescence spectroscopy for the simultaneous determination of sulpiride and mebeverine hydrochloride in their combined tablets and application to real human plasma," *Journal of Fluorescence*, vol. 20, no. 6, pp. 1275–1285, 2010.

[21] R. S. Haggag, R. A. Shaalan, and T. S. Belal, "Validated HPLC determination of the two fixed dose combinations (chlordiazepoxide hydrochloride and mebeverine hydrochloride; carvedilol and hydrochlorothiazide) in their tablets," *Journal of AOAC International*, vol. 93, no. 4, pp. 1192–1200, 2010.

[22] J. Patel, J. K. Patel, and V. P. Patel, "Simultaneous spectrophotometric estimation of mebeverine hydrochloride and chlordiazepoxide in tablet dosage form," *Inventi Rapid: Pharm Analysis & Quality Assurance*, vol. 78, no. 11, 2011, http://inventi.in/journal/article/rapid/4/9277/pharm-analysis-quality-assurance/pi.

[23] H. M. Heneedak, I. Salama, S. Mostafa, and M. El-Sadek, "A stability-indicating HPLC method for the simultaneous determination of mebeverine hydrochloride and chlordiazepoxide in commercial tablets," *Current Analytical Chemistry*, vol. 10, no. 4, pp. 565–573, 2014.

[24] R. Vardanyan and V. Hruby, *Synthesis of Essential Drugs*, Elsevier, Amsterdam, The Netherlands, 1st edition, 2006.

[25] J. A. de Schutter, F. de Croo, G. van der Weken, W. van den Bossche, and P. de Moerloose, "Stability study and quantitative determination of mebeverine hydrochloride in tablets by means of reversed-phase high-performance liquid chromatography," *Chromatographia*, vol. 20, no. 3, pp. 185–192, 1985.

[26] "ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedures: Text and Methodology Q2(R1), International Conference on Harmonization, 2005," May 2013, http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html.

[27] I. A. Naguib and M. Abdelkawy, "Development and validation of stability indicating HPLC and HPTLC methods for determination of sulpiride and mebeverine hydrochloride in combination," *European Journal of Medicinal Chemistry*, vol. 45, no. 9, pp. 3719–3725, 2010.

[28] B. C. Smith, *Infrared Spectral Interpretation: A Systematic Approach*, CRC Press LLC, Boca Raton, Fla, USA, 1999.

[29] J. N. Miller and J. C. Miller, *Statistics and Chemometrics for Analytical Chemistry*, Pearson Education, Harlow, UK, 5th edition, 2005.