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Comparative study of the resolution efficiency of HPLC and HPTLC-densitometric methods for the analysis of mebeverine hydrochloride and chlordiazepoxide in their binary mixture

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ABSTRACT

Accurate, rapid, and selective reversed phase HPLC and HPTLC-densitometric methods with UV detection have been developed and validated for simultaneous determination of a binary mixture of mebeverine hydrochloride (MVH) and chlordiazepoxide (CDZ) in their Co-formulation. For the HPLC method, ACE-126-2546 AQ C-18 column, (250×4.6 mm i.d., 5 µm particle size) in isocratic mode, with mobile phase containing 25 mM ammonium acetate buffer: acetonitrile in the ratio of (60:40, *v*:*v*), pH adjusted to 3±0.2 by using hydrochloric acid, the flow rate of 1.0 mL/min and detection was performed at 260 nm. The retention times were 7.23±0.01 and 3.85±0.01 min for MVH and CDZ, respectively. For the HPTLC-densitometric method, the separation was performed using stationary phase pre-coated silica gel 60F_{254} and mobile phase ethyl acetate: methanol (8:4, *v*:*v*) were used and scanned at 222 nm with Camag TLC scanner controlled by Wincats Software. The R_f values were 0.26±0.02 and 0.73±0.01 for MVH and CDZ, respectively. The linearity graphs for MVH and CDZ, respectively, were found to be linear over 1-50 µg/mL and 0.5-40.0 µg/mL with mean percentage recoveries 100.14±0.354 and 99.70±0.764 for HPLC method and 0.5-30.0 µg/band and 1-14 µg/band with mean percentage recoveries 100.29±0.665 and 99.68±0.987 for HPTLC-densitometric method. A comparative study of different analytical validation parameters such as accuracy, precision, specificity, robustness was conducted. The obtained results were statistically compared with those of the official methods; using student *t*-test, F-test, and one way ANOVA, showing no significant difference with respect to accuracy and precision.

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1. Introduction

Mebeverine hydrochloride, (*RS*)-4-(ethyl[1-(4-methoxy phenyl)propan-2-yl]amino)butyl-3, 4-dimethoxybenzoate hydrochloride (Figure 1) is a musculotropic antispasmodic drug without anticholinergic side-effects. It has a major therapeutic role in the treatment of irritable bowel syndrome (IBS). MVH is also indicated for treatment of gastrointestinal spasm secondary to organic disorder [1]. Chlordiazepoxide, 7-chloro-2-methylamino-5-phenyl-3*H*-1, 4-benzodiazepine-4-oxide (Figure 1) was the first benzodiazepine to be synthesized. CDZ has amnestic, anticonvulsant, anxiolytic, hypnotic and skeletal muscle relaxant properties as it inhibits monosynaptic and polysynaptic reflexes by acting as inhibitory neutral transmitters or by blocking excitatory synaptic transmission [2]. Both drugs have been co-formulated and widely used to reduce irritable bowel syndrome, spastic colon and relief of gastrointestinal manifestation of anxiety and tension of gastrointestinal tract (GIT). Many methods have been reported for the quantitative determination of CDZ based on spectrophotometry [3-5], electrochemical methods [6,7] and chromatographic method [8]. Several analytical procedures have been reported for the quantitative determination of MVH including spectrophotometric methods [9,10], electrochemical methods [6,11] and chromatographic methods [12,13-15].

Few HPLC methods [16,17-20] and spectrophotometry [21] were described for the simultaneous determination of both drugs in solid dosage forms. The survey of literature shows that no HPTLC-densitometric method has been reported for the analysis of the proposed drugs in their co-formulation. So, there is a need for HPTLC-densitometric assay method that permits simultaneous quantification of the proposed drugs and a new HPLC method which can represent another good alternative for the already existing HPLC methods especially when the mobile phases or detectors used for these methods are not present in most of the laboratories.

The aim of this work was to develop and validate RP-HPLC and HPTLC-densitometric methods for resolving this binary mixture.

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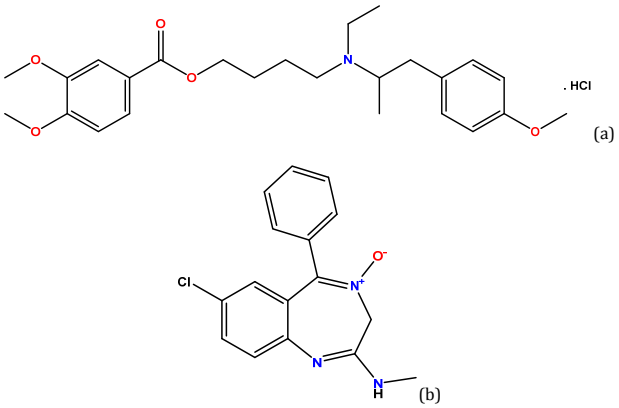

Figure 1. Chemical structure of mebeverine hydrochloride (a) and chlordiazepoxide (b).

The two methods were simple, accurate, precise and robust. A comparative study was conducted between the developed methods to compare between their resolution efficiency and clarify their advantages over each other as well as over the reported ones.

2. Experimental

2.1. Instrumentation

2.1.1. RP-HPLC system

Analysis was performed on a chromatographic system Jasco LC-Net II/ADC (Japan) equipped with UV detector (UV-2070 plus), isocratic pump (PU-2080 plus) and 4-line degasser (DG-2080- 54). A chromatographic separation was achieved by ACE-126-2546 AQ C-18, (250 × 4.6 mm i.d., 5 µm particle size) analytical column. Data acquisition was made with ChromNAV software C.

2.1.2. HPTLC-densitometric system

UV lamp with short wavelength 254 nm (USA), Camag Linomat S auto sampler with Camag micro syringe (100 µL); Camag, Muttenz, Switzerland, Camag TLC-densitometric scanner 3 densitometer model 35/N130319 equipped with wincats software densitometric evaluation; Camag, Muttenz, Switzerland, Pre-coated HPTLC plates (20 cm ×20 cm, 0.25 mm Alugram; NanoSIL Silica Gel G/UV254 Macherey Nagel, Germany). Glass jar for TLC-densitometric with lid 22 × 25 × 10 cm, Source of UVB (Philips Lamp, Germany), 8 watt, with filter producing radiation (280-320). The following requirements are taken into consideration, Slit dimensions: 3×0.45 mm. Scanning speed: 20 mm/s. Spraying rate: 10 s/µL. Data resolution: 100 µm/step.

2.2. Materials

2.2.1. Samples

MVH was kindly supplied by EIPICO, Egypt. Its purity was found to be (99.93±1.57%) according to BP method [6]. CDZ was kindly supplied by EVA Pharmaceutical Company, Egypt. Its purity was found to be (99.45±1.14%) according to BP method [6]. Coloverin®A film-coated tablets were manufactured by Chemipharm Pharmaceutical Industries , 6th October City, Egypt, each tablet contains 135 mg mebeverine HCL and 5 mg chlordiazepoxide (BN 131839A).

2.2.2. Chemicals

For HPLC system, ammonium acetate and hydrochloric acid were supplied from (Adwic-El Nasr Pharmaceutical Chemicals Co. Egypt). Water and methanol (HPLC grade) were purchased by E. Merck, Darmstadt, Germany. Acetonitrile (HPLC grade) was supplied from Lab Scan Limited, Dublin, Ireland. For HPTLC-densitometric system, ethyl acetate and methanol were supplied from (Adwic-El Nasr Pharmaceutical Chemicals Co. Egypt).

2.2.3. Standard solutions

For HPLC system, stock solutions of MVH and CDZ (each, 250 µg/mL) were prepared by dissolving 25 mg of each compound in methanol in 100 mL volumetric flasks then the volume was completed to the mark with the same solvent. All solutions were stored at 4 °C and were stable for 3 months.

For HPTLC-densitometric system, stock solutions of MVH and CDZ (each, 1000 µg/mL) were prepared by dissolving 100 mg of each compound in methanol in 100 mL volumetric flasks then the volume was completed to the mark with the same solvent. All solutions were stored at 4 °C and were stable for 3 months.

2.2.4. Working solutions

2.2.4.1. RP-HPLC method

The dilution was applied using methanol to get working solutions for MVH and CDZ with final concentration (each, 100 µg/mL).

2.2.4.2. HPTLC-densitometric method

The working solutions were prepared as the stock solutions for MVH and CDZ (each, 1000 µg/mL).

2.3. Procedure

2.3.1. RP-HPLC method

RP-HPLC was carried out at ambient temperature on ACE AQ-C_{18} column. The mobile phase consisted of 25 mM ammonium acetate buffer: acetonitrile in the ratio of (60:40, *v:v*) in an isocratic mode. The pH of the mobile phase was adjusted to 3 with 0.1N hydrochloric acid. The mobile phase was filtered using 0.45 µm Millipore membrane filter (Billerica, MA) and delivered at a flow rate of 1 mL/min. The

injection volume was 20 µL and the detection was done at 260 nm.

2.3.1.1. System suitability

Twenty microliters of the working solutions were injected and applied to the chromatographic conditions. The system suitability parameters including retention time, tailing factor, theoretical plate count (N), height of theoretical plate (HETP) and resolution were calculated according to USP guidelines [22].

2.3.1.2. Construction of calibration graphs

Aliquots (1-50 µg/mL) for MVH, (0.5-40.0 µg /mL) for CDZ were transferred from the working solution of each drug (100µg/mL). The corresponding chromatographic conditions were applied for these solutions and the chromatograms were recorded. The calibration graphs of MVH and CDZ were constructed by plotting the relative peak area [the recorded peak area to that of an external standard 10 µg/mL of MVH and CDZ against the corresponding concentration at 264 nm and the regression equations were computed. The linearity graphs for MVH and CDZ, respectively, were found to be linear over the range of 1-50 µg/mL and 0.5-40.0 µg/mL.

2.3.2. HPTLC-densitometric method

HPTLC aluminum sheets 20×10 cm pre-coated with 0.25 mm silica gel. The samples were applied to the HPTLC plate as bands (bandwidth: 6 mm, bands were spaced 1 cm apart from each other and 1 cm from the bottom edge of the plate). The applied volume per band was 10 µL using autosampler with Camag micro syringe (100 µL). The plate was developed to a distance of approximately 8±0.5 cm using mobile phase, ethyl acetate: methanol (80:40, *v*:*v*) in a chromatographic tank previously saturated for 1 h at room temperature. The developed plates were air-dried and scanned at 222 nm. The detection was done using Camage TLC scanner 3 operated in the absorbance mode; with deuterium lamp as a source of radiation; the slit dimension was kept at 3 × 0.45 mm and 20 mm/s scanning speed was employed.

2.3.2.1. System suitability

Parameters including resolution (*R*s) and peak symmetry were calculated for both drugs according to USP guidelines [22].

2.3.2.2. Construction of calibration graphs

For preparation of a calibration graphs, 0.5-30.0 µL of standard working solution of MVH (1000 µg/mL) and 1-14 µL of standard working solution of CDZ (1000 µg/mL) were spotted as bands of 6 mm width on TLC plates. Bands were spaced 1 cm apart from each other and 1 cm from the bottom edge of the plate. Linear ascending plate development to a distance of approximately 8±0.5 cm was performed in a suitable chromatographic tank previously saturated for 1 h with the mobile phase, ethyl acetate: methanol (80:40, *v*:*v*) at room temperature. The developed plates were air-dried and scanned at 222 nm. The chromatograms were recorded. The calibration graphs were constructed by plotting the relative peak area. The peak area found to that of an external standard (6 µg/ mL of MVH and 8 µg/ mL of CDZ) against the corresponding concentrations at 222 nm and the regression equations were calculated. The linearity graphs for MVH and CDZ, respectively, were found to be linear over the range of 0.5-30.0 µg/band and 1-14 µg/band.

2.4. Assay of laboratory-prepared mixtures

Different aliquots of the drugs were accurately transferred from their working solutions and mixed to prepare solutions of different ratios. The chromatographic conditions of both methods were adopted for each laboratory-prepared mixture and the concentrations of each drug were calculated from the corresponding regression equation. Each concentration was conducted from the average of three experiments.

2.5. Application to pharmaceutical dosage form

Twenty tablets of Coloverin®A were accurately weighted and grinded to a fine powder. An amount equivalent to one tablet (containing 135 mg MVH and 5 mg CDZ) was transferred into 100 mL volumetric flask. The powder was extracted with 30 mL methanol for 20 min using vortex shaker and the volume was completed with methanol to obtain a final concentration of 1350 µg/mL of MVH and 50 µg/mL of CDZ, then the solution was filtered through a Whatman No. 10 filter paper (Pore size = 11 µm). From the filtrate, 10 mL was transferred into 100 mL volumetric flasks and the volume was completed with methanol.

For HPLC method, 2 mL was accurately transferred into 10 mL volumetric flask and the volume was completed to the mark with the mobile phase to prepare the working solution to obtain a final concentration 27 µg/mL of MVH and 1 µg/mL of CDZ.

For HPTLC-densitometric method, an appropriate dilution was made with methanol to prepare the working solution to obtain a final concentration 27 µg/band of MVH and 1 µg/band of CDZ.

The corresponding chromatographic conditions were applied for each working solution. Six replicates of each experiment were done. The concentration of each drug was calculated from its corresponding regression equation.

3. Results and discussion

The main task of this work was to develop simple, sensitive and accurate analytical methods for the determination of MVH and CDZ in their binary mixture and pharmaceutical formulation with satisfactory precision for good analytical practice (GAP) and compare between RP-HPLC and TLC- densitometric methods that applied for the determination of MVH and CDZ.

3.1. RP-HPLC method

A simple isocratic high-performance liquid chromatography method was developed for the determination of MVH and CDZ in their binary mixture. Different developing systems with different ratios were tried. It was found that ACE AQ C18 (250 × 4.6 mm) column gave the most suitable resolution for the complete separation of both drugs. To optimize the HPLC assay parameters, the effect of acetonitrile composition and the apparent pH of the mobile phase on the capacity factor (k) were performed.

A satisfactory separation was obtained with a mobile phase consisting of 25 mM ammonium acetate buffer: acetonitrile in the ratio of (60:40, *v*:*v*) after several trials to reach the optimum stationary/mobile-phase matching at pH= 3±0.2 in the room temperature (25±1 °C) and the injection volume was 20 µL. Higher acetonitrile concentration >60% in the mobile phase caused MVH and CDZ peaks to be superimposed and inadequate separation. At lower acetonitrile concentration, the retention time of drug increased, whereas at high or lower pH values resolution was poor. At apparent pH = 2.5 improved resolution of the drug was observed. The drugs MVH and CDZ were scanned by UV, individually, in a wavelength range of 200-400 nm and

maxima for each drug was measured (Figure 2). To optimize the UV maxima, various HPLC experiments were performed at various wavelengths starting from 210 to 350 nm. The best response has achieved with UV detection at 260 nm. The retention times were 7.23±0.01 and 3.85±0.01 mins for MVH and CDZ, respectively (Figure 3). Flow rate 1 mL/min enable acceptable resolution of drug from each other.

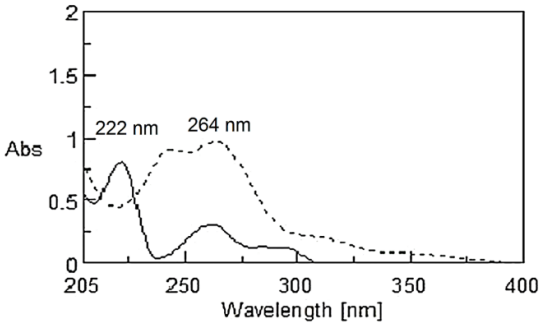

Figure 2. Zero-order spectra of 12 µg/mL of MVH (—) and 10 µg/mL of CDZ (…), separately in methanol

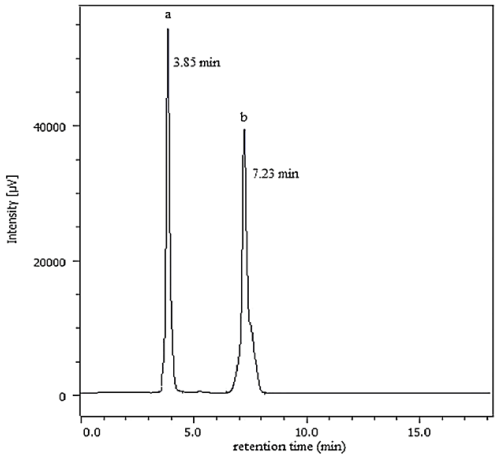

Figure 3. RP-HPLC chromatogram of 5 µg/mL (a) CDZ and 25 µg/mL (b) MVH using C18 (mobile phase consisted of 25 mM ammonium acetate buffer: acetonitrile in the ratio of 60:40 (*v:v*) (pH = 3)), flow rate of 1 mL/min at 260 nm.

3.2. HPTLC-densitometric method

TLC-densitometry is a useful technique for the qualitative and quantitative determination of drug mixtures. This technique offers a simple approach to quantify separated drugs directly on TLC plates via measuring band optical densities. Different band dimensions were tested in order to obtain sharp, symmetrical and well resolved peaks. The optimum band width was chosen (6 mm) and the inter-space between bands was found to be 5 mm. Different scanning wavelengths were tried where 222 nm was found optimum for both drugs. Scanned peaks were sharp, symmetrical and minimum noise was noticed. Moreover, at this wavelength maximum sensitivity was obtained for both drugs. The slit dimensions of the scanning light beam should ensure complete coverage of band dimensions on the scanned track without interference of adjacent bands. Different slit dimensions were tried, where 6 × 0.3 mm proved to be the slit dimension of choice which provides highest sensitivity. It was necessary to investigate the effect of different experimental variables. Different developing systems with different ratios were tried, but the problem was to obtain sharp and compact peak for MVH and CDZ due to tailing upwards and downwards in most of the developing systems. Complete separation was obtained by the system containing ethyl acetate: methanol in the ratio of 80:40 (*v*: *v*). Experimental conditions, such as mobile phase composition, scan mode, speed and detection wavelength were optimized to provide accurate, precise and reproducible results for MVH and CDZ. The chosen scan mode was the zigzag mode and the wavelength of scanning was chosen to be 222 nm. The R_f value was 0.26±0.02 and 0.73±0.01 for MVH and CDZ, respectively. Figure 4 is a scanning profile of the TLC-densitometric chromatogram of 2.5 µg/band MVH and 12 µg/band CDZ, respectively, at 222 nm.

The equilibration time required before development is important to achieve homogeneity of the atmosphere, thus minimizing the evaporation of the solvent from the TLC plate during the development; therefore, the saturation time of the tank has been optimized and found to be 1 h. The plate was developed by ascending chromatography to a distance about 8±0.5 cm with the developed mobile phase. The developed band was visualized under UV lamp at 222 nm.

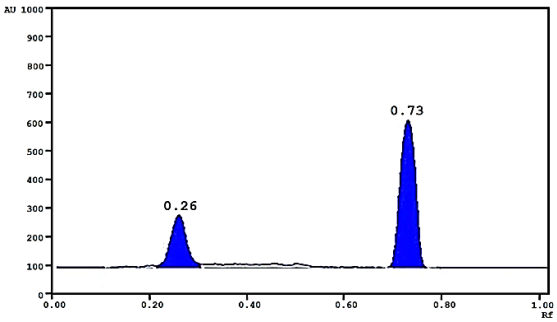

Figure 4. TLC-densitometric chromatogram of 2.5 µg/band MVH and 12 µg/band CDZ, respectively, using ethyl acetate: methanol (8:4, *v:v*) at 222 nm.

3.3. Application to pharmaceutical dosage forms

The suggested HPLC and HPTLC-densitometric methods were valid and applicable for the analysis of MVH and CDZ in Coloverin®A Tablets. The validity of the proposed methods was further assessed by comparing the obtained results with that of the reported method which showed accurate results. The results confirm the suitability of the proposed methods for the routine determination of these components in their combined formulation.

System suitability parameters for both methods were calculated and listed in Table 1. The assay parameters and validation sheet were listed in Table 2. The methods were successfully applied to determine the selected drugs in the laboratory prepared mixtures; and the results were shown in Table 3.

3.4. Methods validation

Method validation was performed for all the proposed methods as follows:

3.4.1. Range and linearity

The linearity of the proposed methods was evaluated by processing the different calibration graphs on three different days. The RP-HPLC method showed higher correlation coefficients (r^2 = 1) than HPTLC-densitometric method (r^2 = 0.9999). The calibration graphs were constructed within concentration ranges that were selected on the basis of the anticipated drugs concentration during the assay of the dosage form.

Table 1. Statistical analysis of parameters required for system suitability of HPLC and HPTLC-densitometric methods.

Parameter	RP- HPLC method		TLC-densitometric method		Reference value [22]
	MVH	CDZ	MVH	CDZ	
t_R (RP-HPLC) R_f (HPTLC-densitometric)	7.23±0.01	3.85±0.01	0.26±0.02	0.73±0.01	
N (Column efficiency)	5559	2932			N > 2000 Increases with efficiency of the separation
HETP (Height equivalent to theor. plates)	0.0045	0.0085			The smaller the value, the higher the column efficiency
T (Tailing factor)	1.279	1.089	1.045	1.05	T = 1 for symmetric peak
Rs (Experimental resolution)	10.071		5.446		Rs >1.5

Table 2. Assay parameters and validation sheet obtained by applying the RP-HPLC and HPTLC-densitometric methods to the binary mixture.

Parameters	RP-HPLC method		HPTLC-densitometric method	
	MVH	CDZ	MVH	CDZ
Linearity range [a]	1- 50	0.5-40.0	0.5-30.0	1-14
Slope	0.0909	0.0958	0.0639	0.0844
Intercept	0.0186	0.0548	0.0837	0.22
Correlation coefficient (r)	1	1	0.99995	0.99995
±SD	±0.35	±0.76	±0.67	±0.98
Mean [a]	100.14	99.70	100.29	99.68
RSD	0.354	0.764	0.665	0.987
Accuracy [b]	100.12±1.191	100.23±1.215	99.48±1.094	100.19±1.025
Intra-day precision [b]	100.89±0.559	100.12±1.215	100.89±0.266	100.03±0.610
Inter-day precision [b]	100.73±0.938	100.31±1.089	100.86±0.325	100.43±0.487

[a] Average of three experiments

[b] Mean value/relative standard deviations (RSD) of three samples.

For RP-HPLC method, calibration graphs were constructed in the range of 1-50 µg/mL for MVH, 0.5-40.0 µg/mL for CDZ. For HPTLC-densitometric method, calibration graphs were constructed in the range of 0.5-30.0 µg/band for MVH and 1-14 µg/band for CDZ. The corresponding assay parameters and validation sheet for the proposed methods were listed in (Table 2).

3.4.2. Accuracy

To study the accuracy of the proposed methods, procedures under linearity were repeated three times for the determination of different blind concentrations of pure drugs. The accuracy expressed as percentage recoveries ±Relative Standard Deviation was shown in Table 2. Good accuracy proved that the excipients in pharmaceutical formulations did not interfere in the analysis of these compounds.

3.4.3. Precision

The precision of the proposed methods, expressed as relative standard deviation (RSD), was determined by the analysis of three different concentrations of pure drugs within the linearity range. The intra-day precision was assessed from the results of three replicate analyses of three pure drugs samples on a single day. The inter-day precision was determined from the same samples analyzed on three consecutive days. Comparing the results of the proposed methods for each mixture, it was found that RP-HPLC method (lower RSD) was more precise than HPTLC-densitometric method. The results were illustrated in (Table 2).

3.4.4. Specificity

The specificity of the methods were investigated by observing any interference encountered from the common tablet excipients such as talc, lactose, glucose, sucrose, starch and magnesium stearate. These excipients did not interfere with the proposed methods. Specificity of the proposed methods was achieved by the analysis of different laboratory prepared mixtures of MVH and CDZ within the linearity range. The RSD showed good percentage recoveries with the lowest standard deviation. Resolution was found to be 10.071 in case of HPLC method and 5.446 in case of HPTLC method, both >1.5 but resolution in HPLC higher than HPTLC. In HPTLC-densitometric method, it becomes difficult to differentiate between overlapping bands and spots. In contrast, the peaks in HPLC can be easily resolved and evaluated by controlling operational parameters such as flow rate of mobile phase, buffer control of the mobile phase, column oven temperature, etc. The good resolution led to satisfactory results for the analysis of the mixtures containing different combination of both drugs as shown in Table 3.

3.5. Robustness

The robustness of the proposed methods was investigated by the analysis of samples under a variety of experimental conditions. For RP-HPLC method, small changes in the pH (±0.2) and small changes in proportions of acetonitrile by up to ±2% were introduced to the mobile phase. A slight change in the retention time and peak parameters was observed however the peak areas were conserved. For HPTLC-densitometric method, small changes in proportions of ethyl acetate by up to ±1% were introduced to the developing system. R_f values and peak symmetry were slightly changed, however the peak areas were conserved. The effect of robustness was more observed in the HPTLC-densitometric method (higher RSD) which proved that RP-HPLC method was more robust upon changing the experimental conditions

3.6. Statistical analysis

Results obtained by the proposed methods for the determination of pure samples of MVH and CDZ, were statistically compared to those obtained by the official methods. The values of the calculated *t*- and F-test were less than the corresponding tabulated ones, which revealed that there was no significant difference with respect to accuracy and precision between the proposed methods and the official ones as shown in Table 4. In order to compare the ability of the proposed methods for the determination of MVH and CDZ, the results obtained by applying the proposed methods were subjected to statistical analysis using one way ANOVA test, there was no significant difference among all of the proposed methods and those obtained by the official methods [6] as shown in Table 5 and the reported method [16] as shown in Table 6.

4. Conclusion

Instrumental planar chromatography with precise application of the samples and computer controlled chromatograms has been considered as reliable for purity control and quanti-

Table 3. Determination of MVH and CDZ in laboratory prepared mixtures by the RP-HPLC and HPTLC-densitometric methods.

Mixture no.	Ratio	RP-HPLC method				HPTLC-densitometric method			
		MVH		CDZ		MVH		CDZ	
		Taken (µg/mL)	Recovery %	Taken (µg/mL)	Recovery %	Taken (µg/band)	Recovery %	Taken (µg/band)	Recovery %
1	2:1	20	99.09	10	99.71	1:1 4	101.44	4	99.72
2	5:1	25	100.39	5	98.00	3:4 6	99.45	8	100.33
3	6:1	30	98.33	5	99.21	1:3 2	98.26	6	98.00
4	1:4	10	100.26	40	100.34	4:5 8	98.00	10	100.90
5	3:2	15	101.31	10	99.71	5:4 10	98.00	8	100.33
Mean±S.D.			99.88±1.17		99.39±0.88		999.03±1.48		99.86±1.12

Table 4. Statistical comparison between the results obtained by the proposed methods and the official BP methods [6] for the determination of MVH and CDZ in pure powder form.

Parameters	RP-HPLC method		HPTLC-densitometeric method		Official BP method [6] [b]	
	MVH	CDZ	MVH	CDZ	MVH	CDZ
Mean	100.14	99.70	100.29	99.68	99.93	99.45
S.D. (±)	0.35	0.76	0.67	0.98	1.57	0.14
No. of experiments	6	6	6	6	6	6
Student's t-test (2.571) [a]	0.7626	0.7246	0.6547	0.7664		
F-test (5.050) [a]	4.2478	2.2234	3.7274	1.3306		

[a] Figures between parentheses represent the corresponding tabulated values of *t*- and F-test at $p = 0.05$.
[b] BP methods for MVH and CDZ are a non-aqueous potentiometric titrimetric methods. British Pharmacopoeia, Vol. I and II, The Stationery Office on Behalf of Medicines and Healthcare Products Regulatory Agency (MHRA), 2013. [6]

Table 5. One way ANOVA testing for the different proposed and the official methods used for the determination of MVH and CDZ in pure powdered form *.

Compound	Source	DF	Sum of squares	Mean square	F value	F critical
MVH	Between exp.	2	0.384	0.192	0.189	3.682
	Within exp.	15	15.166	1.011		
CDZ	Between exp.	2	0.223	0.111	0.118	3.682
	Within exp.	15	14.190	0.946		

* At the $p = 0.05$ level, the population means are not significantly different.

Table 6. One way ANOVA testing for the different proposed and the reported method [16] used for the determination of MVH and CDZ in their dosage form *.

Compound	Source	DF	Sum of squares	Mean square	F value	F critical
MVH	Between exp.	2	1.062	0.531	4.307	5.143
	Within exp.	6	0.740	0.123		
CDZ	Between exp.	2	4.234	2.117	4.349	5.143
	Within exp.	6	2.919	0.487		

* At the $p = 0.05$ level, the population means are not significantly different.

tative drug testing. Most of the reported mobile phases were of relatively complex composition. Thus, the aim of this TLC-densitometric work was to investigate the use of new, simple, two component only mobile phase. Different developing systems of different composition and ratios were tried for separation and results were evaluated with respect to efficiency of separation and the shape of separated bands. This mobile phase allowed good separation between the binary mixtures with good R_f values without tailing of the separated bands.

Comparing the RP-HPLC and HPTLC-densitometric methods for analysis of binary mixture of MVH and CDZ, it was found that the proposed methods provided accurate, sensitive and selective quantitative analysis of a mixture in bulk powder, laboratory-prepared mixtures and dosage form. The HPTLC-densitometric method had the advantages over the proposed RP-HPLC method, of being with minimal sample clean-up, wide choice of mobile phases, flexibility in sample distinction, high sample loading capacity and low cost (inexpensive apparatus and solvents) and time, as up to 20 samples could be applied to a single plate and analyzed per one development. The specificity of the HPLC method is excellent, high resolution factor, powerful, adaptable, automated process and simultaneously sufficient precision is also attainable. However, it has to be stated that the astonishing specificity, precision and accuracy are attainable only if wide-ranging system suitability tests are carried out before the HPLC analysis. For the reason of the expense that be paid for high specificity, the proposed RP-HPLC applied a simple isocratic mobile phase, unlike the gradient reported method in which, the mobile phase ratio and composition seemed rather critical; so the robustness of the reported method could therefore be significantly affected. The proposed RP-HPLC method differed from the other reported methods through using different conditions which lead to consuming smaller amounts of solvents on the large scale (quality control laboratories) and saving the life time of the column used. The co-formulated tablets were determined using the regression equations method. The results obtained were statistically compared with the reported HPLC method using *t*- and F-tests at 95% confidence level, showing no significant difference with respect to accuracy and precision

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