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Quantitative determination of two anti-hypertensive drugs in their combined dosage form by spectrophotometric and high pressure liquid chromatographic methods

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ABSTRACT

In this study determination of Telmisartan (TELM) and hydrochlorothiazide (HCT) were conducted by application of Spectrophotometry and Hi-Performance-Liquid-Chromatography (HPLC). Four different accurate, sensitive and reproducible methods were applied for the simultaneous determination of (TELM) and (HCT) in their bulk powder and pharmaceutical dosage form. The first method is multi-wavelength analysis in which two wavelengths were selected for each drug in such a way that the difference in absorbance was zero for the second drug. At wavelengths 274.2 nm and 308.6 nm TELM had equal absorbance values; therefore, these two wavelengths have been used to determine HCT; on a similar basis 300 nm and 321 nm were selected to determine TELM. The second method is Q-analysis (graphical absorbance ratio) method involve the formation of Q-absorbance equation using the respective absorptivity values at 280 nm (ISO-absorptive point) and 274 nm (λ max of HCT). The third method is mean centering of ratio spectra (MCR) with measurement at 302.2 nm and 338.8 nm for TELM and HCT respectively. The fourth method is an isocratic reversed-phase HPLC procedure using a mobile phase 0.05M ammonium acetate (pH 6.0): acetonitrile: methanol (30:45:25) with detection at 274 nm on an CSC-INERTSILODS C18 column (5 um particle size, 150 mm length x 4.6 mm internal diameter) at a flow rate 1.2 ml/min. The suggested procedures were checked using laboratory prepared mixtures and were successfully applied for the analysis of their pharmaceutical preparations. The validity of the proposed methods was further assessed by applying the standard addition technique. The results obtained by applying the proposed methods were statistically analyzed and compared with a reported method.

KEYWORDS

Telmisartan;
Hydrochlorothiazide;
Multi-wavelength;
Graphical absorption ratio;
Mean centering of ratio spectra;
RP-HPLC methods.

INTRODUCTION

TELM is chemically, 2-(4-{[4-methyl-6-(1-methyl-1*H*-1,3-benzodiazol-2-yl)-2-propyl-1*H*-1,3-benzodiazol-1yl]methyl}phenyl)benzoicacid, Figure (1). TELM is an angiotensin II receptor antagonist (more

commonly called an "ARB", or angiotensin receptor blocker) act by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland without affecting other systems involved in cardiovascular regulation By blocking the vasoconstrictor and aldosterone secreting effects of angiotensin II. TELM dilates blood vessels and reduces blood pressure[1]. Unlike ACE inhibitors, they do not inhibit the breakdown of bradykinin and other kinins, and thus are unlikely to cause the persistent dry cough which commonly complicates ACE inhibitor therapy. They are therefore a useful alternative for patients who have to discontinue an ACE inhibitor because of persistent cough. TELM is official in British pharmacopoeia[2].

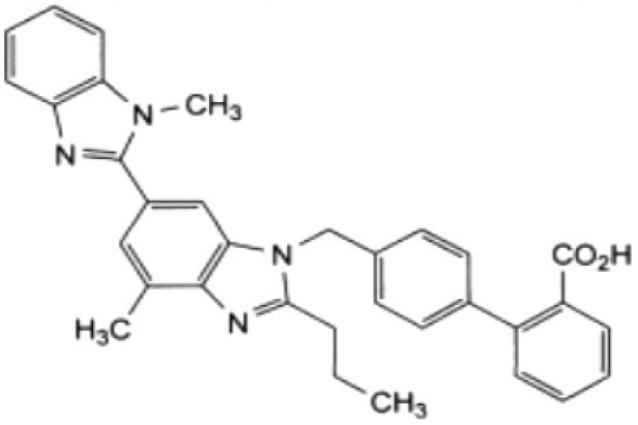

Figure 1 : Chemical structure of TELM

Hydrochlorothiazide is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide, Figure (2). HCT is a first-line diuretic drug of the thiazide class that acts by inhibits active chloride reabsorption at the early distal tubule via the Na-cl co-transporter, resulting in an increase in the excretion of sodium, chloride, and water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance. HCT is a calcium-sparing diuretic, meaning it can help the body get rid of excess water while still keeping calcium[1]. HCT is official in British pharmacopoeia[2] and in USP pharmacopoeia[3].

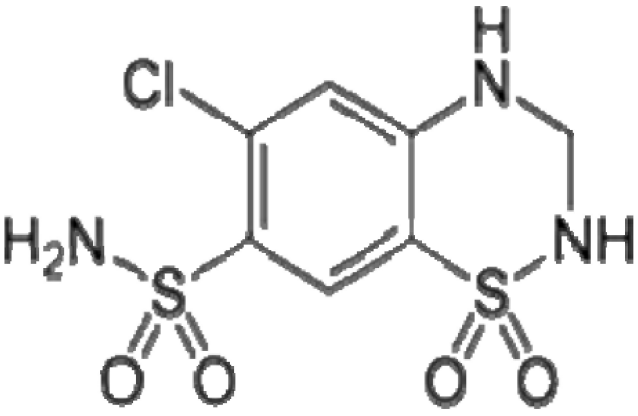

Figure 2 : Chemical structure of HCT

Several analytical procedures have been proposed for the quantitative estimation of TELM and HCT separately and in combination with other drugs. Linear sweep polarography[4], HPLC[5], and UV[6] methods for estimation of TELM alone in pharmaceutical preparation have been reported. Hydrochlorothiazide in combination with other drugs is estimated by HPLC[7], LC and HPTLC-densitometry[8], capillary electrophoresis, capillary electro-chromatography[9] and spectrophotometric methods[10]. Simultaneous estimation of TELM and HCT has been reported by RP-HPLC[11,12], and by TLC-DENSITOMETERIC and derivative spectrophotometry[14]. To our knowledge simple and economical analytical method for simultaneous determination of TELM and HCT has not been reported so far. In this paper four new, simple methods for simultaneous estimation of TELM and HCT in tablet formulation were developed.

EXPERIMENTAL

Apparatus

- SHIMADZU UV-1650 PC, dual beam UV–visible spectrophotometer with two matched 1 cm quartz cells, connected to an IBM compatible personal computer (PC) and an HP-600 inkjet printer. Bundled UV-PC personal spectroscopy software version (2.21) was used to process the absorption and the derivative spectra. The spectral band width was 0.2 nm with wavelength scanning speed of 2800nm min^{-1}.
- Ratio spectra were mean centered using MATLAB 7.0 software.
- Chromatographic system used was Agilent-1100 series comprised of degasser, quaternary pump, auto injector, column compartment, an ultraviolet variable wavelength detector (model G1314A, agilant1100 series) and the system was controlled

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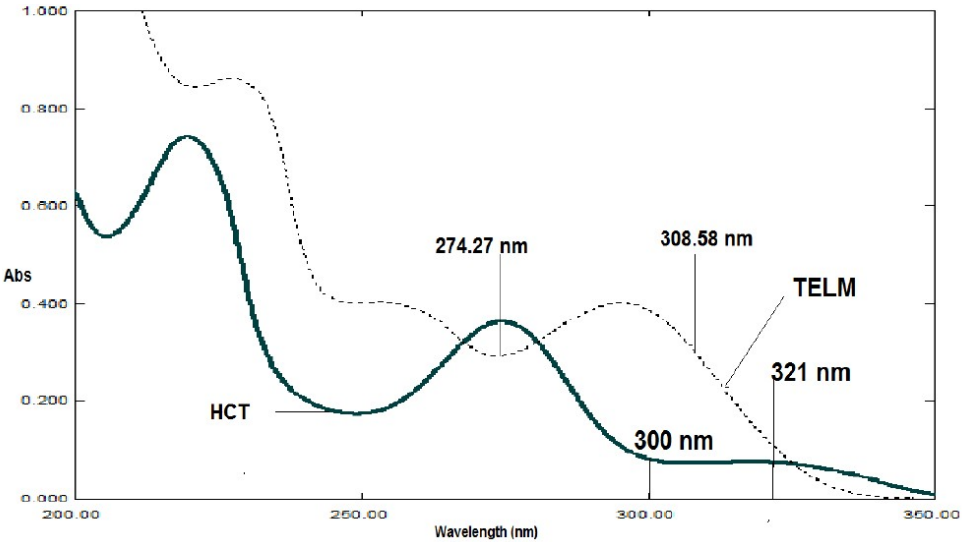

Figure 3 : Zero order absorption spectra of TELM and HCT (each of 6 ug/ml) showing selection of four wavelengths (274.27 nm, 308.58 nm for HCT) and (300nm, 321 nm for TELM) for multi-wavelength method

through CHEMSTATION SOFTWARE, stationary phase is an CSC- INERTSIL ODS C18 column (5um, 4.6x150mm internal diameter) analytical column.

Materials

Pure samples

Standard TELM and HCT were kindly supplied by ALKAN PHARMA, Cairo, Egypt and used without further purification. Purity was determined by reported HPLC method[12] and found to be 99.95 ±0.6 and 100.02 ± 0.4 respectively.

Market samples

MICARDIS TABLET (B.N 100251) labeled to contain 40 mg TELM and 12.5 mg HCT was procured from the local market.

Chemicals and reagents

All reagents and solvents used throughout this work were of analytical pure grade.

- Double distilled water
- Acetonitrile, Methanol are HPLC- grade; E. Merck (Darmstadt, Germany).
- Ammonium acetate, acetic acid, sodium hydroxide, were HPLC grade– (El Nasr Pharmaceutical & Chemical Co. Egypt)

Standard solutions

Stock solutions

Standard stock solution of Telmisartan and Hydrochlorothiazide was prepared by dissolving 10 mg of each drug separately in 10mL volumetric flask using 0.1N sodium hydroxide as solvent. Stock solutions of 1000 µg/mL were obtained in this manner.

Working solutions

Working standard solutions of concentration 100 µg/mL each were prepared by appropriate dilutions of stock solutions by distilled water.

Laboratory-prepared mixtures

For spectrophotometric methods: Accurate aliquots equivalent to 2–20 μgmL^{-1} of TELM were transferred from its working standard solution (100 μgmL^{-1}) into a series of 10-mL volumetric flasks, followed by aliquots equivalent to 2–20 μgmL^{-1} of HCT from its working standard solution (100 μgmL^{-1}) and the volume diluted to mark with distilled water and mixed well.

For HPLC method: aliquots equivalent to 1-32 μgmL^{-1} both drugs were transferred into a series of 10-ml volumetric flasks, from their working standard solution (100 μgmL^{-1}) and the volume diluted to mark with mobile phase and mixed well.

PROCEDURES

Spectrophotometric methods

Method I (Multi-wavelength method)

Standard solutions of both TELM and HCT in the

range of 2-20 and 2-20 μgmL^{-1}, respectively were separately prepared by appropriate dilutions of their respective working standard solutions in distilled water and then scanned in the range of 200-400 nm. Absorbance values at 300 nm and 321 nm (for TELM) and at 274.2 nm and 308.6 nm (for HCT) were measured. TELM was determined by plotting the difference in absorbance at 300 nm and 321 nm (difference is zero for HCT) against its corresponding concentration. Similarly for determination of HCT, the difference in absorbance at 274.2 nm and 308.6 nm (difference is zero for TELM) was plotted against the corresponding concentration. The concentrations of the two drugs were calculated each from the corresponding calibration curve equation.

Method II (Q-analysis method)

Standard solutions containing 2-20 μgmL^{-1} of TELM and 2-20 μgmL^{-1} of HCT were prepared separately using distilled water methanol as a solvent. The absorption spectra of the prepared solutions were recorded in the range of 200-400 nm and the absorbance values at 280 nm (λ ISO) and 274 nm (λ max of HCT) were measured from which the absorptivity values for both drugs at these selected wavelengths were calculated. The method employs Q values and the concentrations of the studied drugs in the prepared solutions were determined by using the following equations:

$$C_X = [Q_m - Q_y / Q_x - Q_y] \times A_1 / A_{x1}$$

$$C_Y = [Q_m - Q_x / Q_y - Q_x] \times A_1 / A_{y1}$$

Where C_x and C_y are the concentrations of TELM and HCT in μgmL^{-1}, respectively; Q_m is the absorbance of the sample at λ_{274}/ absorbance of sample at λ_{280}, Q_x is the absorptivity of TELM at λ_{274}/ absorptivity of TELM at λ_{280}; Q_y is the absorptivity of HCT at λ_{274}/ absorptivity of HCT at λ_{280}; A_{x1} is the absorptivity of TELM at $_{280}$; A_{y1} is the absorptivity of HCT at λ_{280}; and A_1 is the absorbance of the Mixture at λ_{280}.

Method III (Mean centering of ratio spectra method)

Standard solutions of TELM equivalent to 2-20 μgmL^{-1} were accurately transferred from its standard working solution (100μgmL^{-1}) into a set of 10 mL measuring flasks and the volume was adjusted by distilled water. The absorption spectra of the prepared solutions were recorded in the range of 250-350 nm, divided by the standard spectrum of 2 μgmL^{-1} of HCT and then the obtained ratio spectra were mean centered. By the same way the spectra of different concentrations of standard solutions of HCT in the range of 2-20 μgmL^{-1} were recorded. The stored spectra were divided by the standard spectrum of 2 μgmL^{-1} of TELM to obtain the ratio spectra which were then mean centered. Calibration curves for both TELM and HCT were constructed by plotting the amplitude values of their respective mean centered ratio spectra at 302.2 nm and 338.8 nm respectively against their corresponding concentrations.

HPLC method

Chromatographic conditions

(1) CSC-INERTSIL ODS C18 column (5 um particle size, 150 mm length x 4.6 mm internal diameter).
(2) Mobile phase: 0.05M ammonium acetate (pH 6.0): acetonitrile: methanol (30:45:25 v/v/v). (The mobile phase was filtered using 0.45μm membrane filter and degassed by ultrasonic vibrations for 15 min.)
(3) Flow rate was 1.2 ml/min. at 40°C.
(4) Detector wavelength: 274 nm.
(5) Runtime: 5.5 min
(6) Injection volume: 10μl.

Construction of calibration curve

Aliquots of both TELM and HCT working standard solution (100μgmL^{-1}) equivalent to 1-32 μgmL^{-1} were accurately transferred into separate series of 10-ml volumetric flasks, volume was completed with mobile phase. The prepared samples were analyzed using the previously mentioned chromatographic conditions. A calibration curve relating the peak area ratios of each drug versus their corresponding concentration were constructed and the regression equations were computed.

Application in tablet dosage forms

Twenty tablets were weighed and the average weight was calculated and crushed to a fine powder. An accurately weighed powder sample equivalent to 100 mg of TELM was transferred to a 100 ml volumetric flask, dissolved in 10 ml sodium hydroxide, shaken for 10 min and the volume was made up to the

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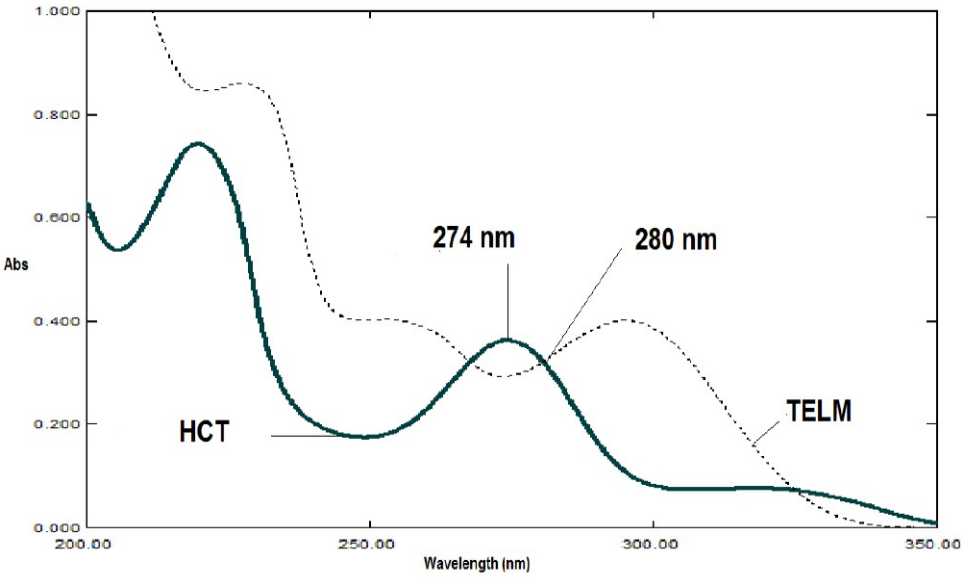

Figure 4 : Zero order absorption spectra of TELM and HCT (each of 6 ug/ ml) showing selection of two wavelengths (HCT λmax = 274 nm, λiso = 280 nm) for Q-absorption ratio method

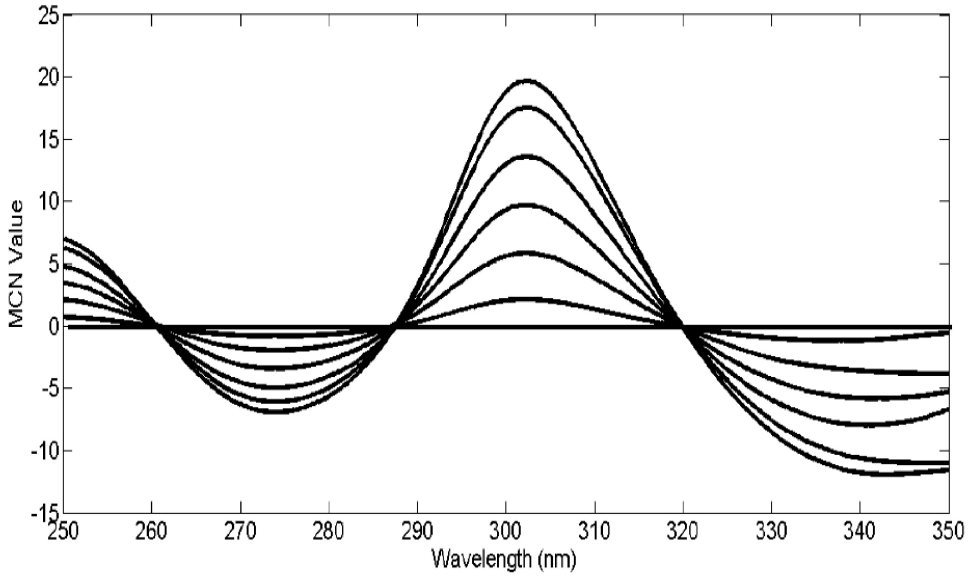

Figure 5 : Mean centered ratio spectra of TELM (2-20 ug/ml) using 2ug/ml of HCT as a divisor and distilled water as a blank

mark with distilled water. The solution was then filtered by WHATMAN filter paper no 41. The solution was diluted to the same concentrations of working standard solutions and the general procedures under linearity were followed. The validity of the methods was assessed by applying the standard addition technique.

RESULTS AND DISCUSSION

Spectrophotometric methods

Multi-wavelength method

The developed multi-wavelength method provides a simple method for selective determination of both TELM and HCT using their zero order absorption spectra. The principle of this method is that the absorbance difference at two points on the spectra is directly proportional to the component of interest, independent on the interfering component[15-17]. The pre-requisite for this method is the selection of two wavelengths where the interfering component shows the same absorbance value while the component of interest shows significant difference in absorbance with concentration[15]. Different wave lengths were tried. Using the absorbance values at 300 nm and 321 nm where HCT has the same

absorbance gave the best selectivity when used for determination of TELM. On the other hand, the absorbance values at 274.2 nm and 308.6 nm where chosen for determination of HCT where the best results were obtained, Figure (3). Calibration curves for TELM and HCT were constructed by plotting the difference in absorbance values at the selected wavelengths for each drug against their corresponding concentrations. TELM and HCT both obeyed Beer Lambert's Law in the concentration ranges of 2-20 ug mL^{-1} with good correlation coefficients. Regression equation parameters are given in TABLE (4).

Q-analysis (graphical absorbance ratio) method

This method depends on the property that for the substance that obeys Beer's Lambert's law at all wavelengths, the ratio of absorptivity (or absorbance) values at any two wavelengths are constant, independent on the concentration or path length. This ratio is referred as Q-ratio. One of the two selected wavelengths is an (ISO-absorptive point and the other is the wavelength of maximum absorption of one of the two components[18-22]. From the overlain spectra of the two drugs and their mixture, Figure (4), it is evident that TELM and HCT show ISO-absorptive point at 280 nm; TELM has λ max at 295 nm while HCT has λ max at 274 nm. Using the absorbance values at 280 nm (λiso) and 274 nm (λ max for HCT) gave the best results regarding selectivity. The absorbance values at 280 nm and 274 nm for both TELM and HCT in the range of 2-20 μgmL^{-1} were obtained. Absorptivity coefficients were determined for both drugs and the average values were taken. The absorptivity values and the absorbance ratio were used to develop the following sets of equations from which the concentration of each component in the sample can be calculated:

$$C_{TELM} = [Q_m - Q_y / Q_x - Q_y] \times A_1 / A_{x1}$$

$$C_{HCT} = [Q_m - Q_x / Q_y - Q_x] \times A_1 / A_{y1}$$

Where C_{TELM} is the concentrations of TELM in μgmL^{-1}; C_{HCT} is the concentrations of HCT in μgmL^{-1}; Q_m is the absorbance of sample at λ_{274} /absorbance of sample at λ_{280}; and A_1 is the absorbance of the sample at λ_{280}.

Mean centering of ratio spectra spectrophotometric method (MCR)

To optimize the developed MCR method [23-25], different parameters were tested. Since the wave length range taken has a great effect on the obtained mean centered ratio spectra, different wave length ranges were tested and the best results were obtained upon using wave length ranging from 250-350 and 280-380 nm for TELM and HCT, respectively. The effect of divisor concentration on the selectivity was checked by testing several concentrations. The best results regarding sensitivity and selectivity were obtained upon using 2 μgmL^{-1} of HCT and 2 μgmL^{-1} of TELM as divisors. To construct the calibration curves of the proposed method,

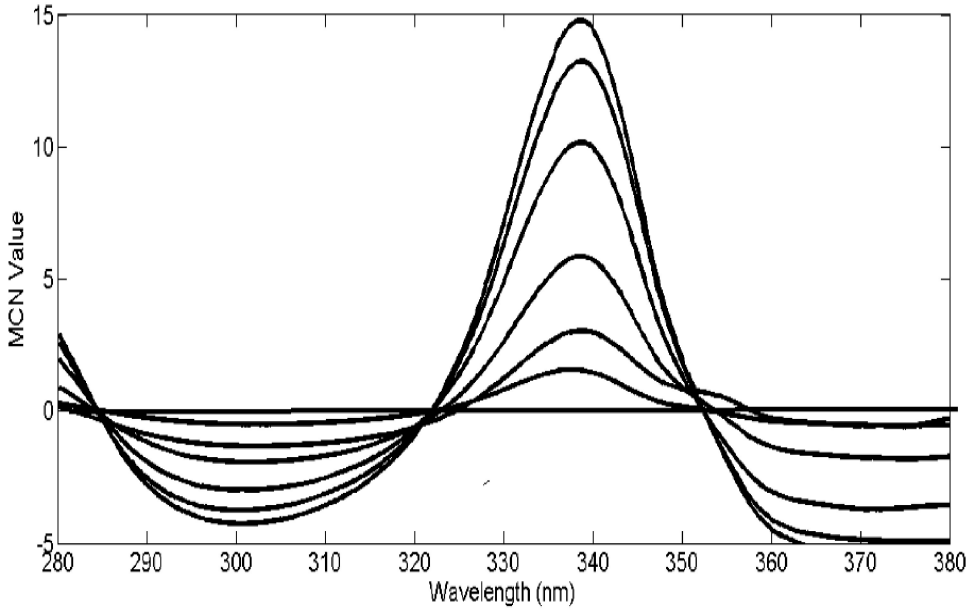

Figure 6 : Mean centered ratio spectra of HCT (2-20 ug/ml) using 2ug/ml of TELM as a divisor and distilled water as a blank

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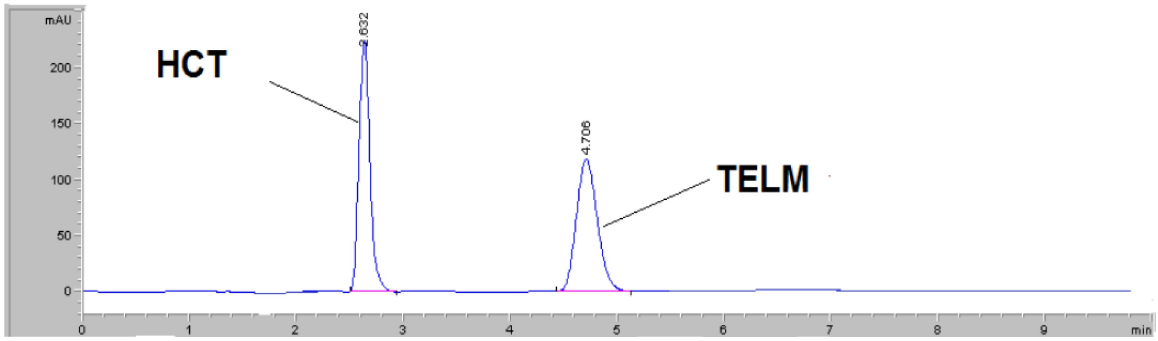

Figure 7 : HPLC chromatogram showing HCT (2.632 min) and TELM (4.706 min)

the absorption spectra of the standard solutions of TELM with different concentrations were recorded in the wave length range from 250-350 nm and divided by the standard spectrum of HCT (2 μgmL^{-1}). Then, mean centering of the resulted ratio spectra has been obtained and the concentrations of TELM were determined by measuring the amplitude value of the mean centered ratio spectra at 302.2 nm as shown in Figure (5). By the same way, different standard solutions of HCT with different concentrations were recorded and divided by the standard spectrum of TELM (2 μgmL^{-1}) and the ratio spectra were obtained which were then mean centered. The amplitude value at 338.8 nm in the obtained mean centered ratio spectra were used for determination of HCT as shown in Figure (6). The computed regression parameters for each of the studied drugs are given in TABLE (4).

HPLC method

A high-performance liquid chromatographic method is described for the quantitative determination of TELM and HCT in bulk materials and in pharmaceutical formulations. All experimental conditions were investigated to optimize the proposed HPLC method. Different columns were investigated; CSC-INERTSIL ODS C18 column (5 um particle size, 150 mm length x 4.6 mm internal diameter) was chosen which gave best resolution. While for mobile phase, different systems were tried for chromatographic separation of TELM and HCT. The best resolution was achieved using a mobile phase consisting of 0.05M ammonium acetate (pH 6.0): acetonitrile: methanol (30:45:25 v/v/v). The retention time were found to be 4.706 and 2.632 min for TELM and HCT, respectively, Figure (7). System suitability was checked by calculating the resolution, tailing factor, column efficiency (N). HETP, and the separation factor, where the system was found to be suitable indicating good resolution of the components, TABLE (2).

The proposed methods were also applied for the determination of the two drugs in their dosage forms and the results obtained were shown in TABLE (3). Assay validation parameters are presented in TABLE (4). The proposed methods were compared statistically with the reported method using phosphate buffer (pH: 3.5): acetonitrile (50:50 v/v) as a mobile phase [12] and no significant difference was obtained as shown in TABLE (5).

METHODS VALIDATION

Validation of the method has been carried out according to ICH guidelines[26].

Linearity and range

The calibration range for TELM and HCT were established through considerations of the practical range necessary according to adherence to Beer-Lambert's law and the concentration of TELM and HCT present in the pharmaceutical dosage form to give accurate, precise and linear results. Linearity ranges of both TELM and HCT are shown in TABLE (4).

Accuracy

The accuracy of the results was checked by applying the proposed methods for determination of different blind samples of TELM and HCT and the concentrations were obtained from the corresponding regression equations. Good percentage recoveries were obtained and were presented in TABLE (4). Accuracy of the methods was further assured by applying the standard addition technique where good results were obtained, confirming the accuracy of the proposed methods, TABLE (3).

TABLE 1 : Determination of the studied drugs in the laboratory prepared mixtures by the proposed methods

mix. no	Ratio TELM; HCT	Taken amount for spectrophotometric methods		Taken amount for HPLC methods		Found recovery %							
						Multi wavelength		Q-analysis		MCR		HPLC	
		TELM	HCT	TELM	HCT	TELM	HCT	TELM	HCT	TELM	HCT	TELM	HCT
1	1 : 1	4	4	24	24	99.50	100.25	99.25	100.75	98.75	101.00	100.38	99.63
2	2 : 1	12	6	24	12	100.67	99.00	100.58	98.83	100.33	99.33	100.92	102.00
3	1 : 2	6	12	12	24	99.33	99.58	100.67	98.83	99.50	100.17	98.67	100.96
4	3.2 : 1*	16	5	32	10	99.19	100.40	99.06	98.80	100.31	101.20	99.81	98.90
5	1 : 3.2	5	16	10	32	99.40	100.50	100.40	100.81	99.60	99.88	100.50	100.47
6	3 : 1	12	4	24	8	100.92	99.25	101.25	99.00	100.83	99.50	99.46	100.88
7	1 : 3	4	12	8	24	100.50	98.75	101.75	99.42	100.50	98.58	102.00	101.88
8	1 : 1	10	10	32	32	99.00	99.80	99.30	100.90	101.50	99.60	99.53	101.13
		Mean ± S.D				99.81 ± 0.754	99.69 ± 0.660	100.28 ± 0.991	99.67 ± 0.975	100.17 ± 0.858	99.91 ± 0.869	100.16 ± 1.025	100.73 ± 1.055

***Corresponding to their ratio in pharmaceutical formulation**

TABLE 2 : Parameters required for system suitability test for HPLC method

Parameters	TELM	HCT	Reference value[27,28]
Resolution (R)	7.47	-	R > 0.8
Relative retention (α) time (selectivity)	1.79	-	>1
Number of theoretical plates (N)	2641	3134	increases with efficiency of the separation
Tailing factor (T)	0.92	0.86	T= 1 for a typical symmetric peak
(HETP)	0.006	0.005	The smaller the value, the higher the column efficiency

TABLE 3 : Determination of TELM and HCT in their pharmaceutical formulation and application of standard addition technique

Pharmaceutical preparation	Multi wavelength		Q-analysis		MCR		HPLC	
	TELM	HCT	TELM	HCT	TELM	HCT	TELM	HCT
MICARDIS® tablets (CONTAIN 40 MG TELM+12.5 HCT) B.N 100251	99.96 ± 0.861	99.39 ± 0.923	100.08 ± 1.126	99.77 ± 1.041	100.06 ± 0.962	100.09 ± 1.087	100.13 ± 1.118	99.59 ± 1.368
Standard addition technique Mean ± S.D	99.69 ± 0.925	99.94 ± 1.005	100.42 ± 1.234	99.43 ± 1.15	99.93 ± 1.102	99.60 ± 1.311	100.80 ± 1.271	99.65 ± 1.443

a Average of six determinations; b Average of three determinations

Precision

Repeatability

Three concentrations of TELM and HCT (8, 10, 12 μgmL^{-1}) were analyzed three times intra-daily using the proposed methods. Acceptable RSD% values were obtained, confirming the repeatability of the methods, TABLE (4).

Intermediate precision

The previous procedures were repeated inter daily on three different days for the analysis of the three chosen concentrations and RSD% values were calculated, TABLE (4).

Specificity

To test the selectivity of the developed methods,

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TABLE 4 : Assay validation of the proposed methods for the determination of TELM and HCT

Parameters	Multi wavelength		Q-analysis		MCR		HPLC	
	TELM	HCT	TELM	HCT	TELM	HCT	TELM	HCT
λ (nm)	Difference in absorbance between 300 and 321 nm	Difference in absorbance between 274 and 308 nm	280 nm (λiso) and 274 nm (λmax for HCT)		Peak amplitude 302.2 nm	Peak amplitude 338. nm	274 nm	
Beers law range	2-20 ug/ml	2-20 ug/ml	2-20 ug/ml	2-20 ug/ml	2-20 ug/ml	2-20 ug/ml	2-32 ug/ ml	2-32 ug/ ml
Regression equation	Y=0.0454X+ 0.0024	Y=0.052X+ 0.0029	C=(Qm-1.181/0.941-1.181) XA1/0.0547	C=(Qm-0.941/1.181-0.941) xA1/0.0547	Y= 0.9726X+0.0594	Y= 0.7322X+0.0428	Y= 4335.1X+574.92	Y= 9321.4X-745.53
Correlation Coefficient	0.9996	0.9999	…..	…..	0.9998	0.9998	0.9996	0.9995
Accuracy mean± S.D	99.72 ± 0.553	100.25 ± 0.614	99.04 ± 0.845	100.71 ± 0.847	99.86 ± 0.587	99.91 ± 0.869	100.69 ± 1.074	99.44 ± 0.999
LOD* (μgmL^{-1})	0.53	0.28	0.53 & 0.35	0.56 & 0.35	0.40	0.39	0.33	0.32
LOQ* (μgmL^{-1})	1.62	0.84	1.62 & 1.07	1.71 & 1.07	1.22	1.19	0.99	0.98
Precision								
Repeatability	0.568	0.86	0.258	0.351	0.370	0.328	0.621	0.567
Intermediate precision	0.942	0.86	0.430	0.554	0.516	0.560	0.857	0.834

***Limit of detection and quantitation were determined by; LOD = (SD of response/slope) X 3.3, LOQ = (SD of response/slope) X 10.**

TABLE 5 : Statistical analysis of the results obtained by the proposed methods and the reported method

parameters	Multi -wavelength method		Q-analysis		MCR		HPLC		Reported HPLC method**	
	TELM	HCT	TELM	HCT	TELM	HCT	TELM	HCT	TELM	HCT
Mean	99.72	100.25	99.04	100.71	99.86	99.91	100.69	99.44	99.65	100.24
S.D	0.553	0.614	0.845	0.847	0.587	0.869	1.074	0.999	1.232	1.134
N	6	6	6	6	6	6	6	6	5	5
Variance	0.306	0.377	0.714	0.717	0.345	0.755	1.153	0.998	1.518	1.286
Student´s t-test [C] (2.262)	0.117	0.018	0.938	0.765	0.349	0.533	1.477	1.231	-	-
F-value [C] (5.19)	4.961	3.411	2.126	1.794	4.400	1.703	1.317	1.289	-	-

C The value in the parenthesis are the corresponding theoretical values of t and F at (p= 0.05); ** Reported RP-HPLC method using phosphate buffer (pH: 3.5): acetonitrile (50:50 v/v) as a mobile phase with UV detection at 271 nm

they were applied for analysis of number of laboratory prepared mixtures containing TELM and HCT in different ratios within their linearity ranges. The good percentage recoveries and low RSD% values shown in TABLE (1), confirming the high selectivity of the suggested methods.

CONCLUSION

Four simple, specific and accurate methods have been developed and validated for simultaneous determination of TELM and HCT in pure form, laboratory prepared mixtures and combined dosage form. The developed multi-wavelength analysis and Q-method differ from the reported spectrophotometric ones in using zero order absorption spectra and no derivetization, so signal to noise ratio is enhanced. Also they are time consuming and cost-effective. Moreover, the developed MCR method has advantages over the published methods in being more simple, rapid and data processing step is not time consuming as it does not need the application of complex algorithms. Also, it does not need derivative steps and so signal to noise ratio has enhanced. On the other hand, the developed RP-HPLC method

has advantage over other published method in using 15 cm column which save 40% of solvent and 40% of time (runtime of 5.5 min) compared to other published method that use 25 cm column. Moreover, TELM is very lipophilic drug and so operating at high (pH 6.0) enables (-COOH of TELM) to give carboxylate anion ($-COO^-$) so it become less lipophilic and elute more rapidly, and also the developed RP-HPLC use a mixture of acetonitrile and methanol as organic modifier and therefore decrease the percentage of acetonitrile which is very expensive and toxic solvents compared to methanol.

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