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ORIGINAL ARTICLE

Development and Validation of Successive Ratio Derivative Spectra Method for Simultaneous Estimation of Telmisartan, Chlorthalidone and Cilnidipine in Tablet Dosage Form

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ABSTRACT
A new method was developed for the simultaneous quantification of ternary mixtures by successive ratio derivative spectra method and validated as per International Conference on Harmonization [(ICH) Q2 (R1)] guideline. Methanol used as solvent for this method. Calibration graphs were established in the range 10-50 µg/mL for Telmisartan, 7.5-17.5 µg/mL for Chlorthalidone and 5-25 µg/mL for Cilnidipine. All the drugs exhibited good linearity over the reported concentration range with acceptable correlation coefficient. The method was validated according to ICH guideline parameters such as accuracy, repeatability, reproducibility showing acceptable percent relative standard deviation of less than 2. The proposed method demonstrated that the method is accurate, simple, precise, compounds not interference with each other and no any prior separation of compounds.
Keywords: *Telmisartan (TEL), Chlorthalidone (CHL), Cilnidipine (CIL) and Successive ratio derivative spectra method*

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INTRODUCTION

Telmisartan (TEL) is chemically nominated as (4-{[4-Methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl) benzoic acid (Fig.1a.). Its molecular formula is $C_{33}H_{30}N_4O_2$. It is an angiotensin receptor blocker that shows high affinity for the angiotensin II type 1 (AT1) receptors [1]. Telmisartan (TEL) shows comparable antihypertensive - activity to other major antihypertensive classes, such as angiotensin converting enzyme (ACE) inhibitors, beta-blockers, and calcium antagonists [2]. Chlorthalidone (CHL) is chemically known as [2-chloro-5-(1-hydroxyl-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide] (Fig.1b.). It is a thiazide-like diuretic, as it acts in a similar manner to the thiazide drugs but does not include the benzothiadiazine structure. It is used in the treatment of fluid retention caused due to kidney disease and hypertension by reducing the electrolyte salts and water in the body. It also used in the treatment of diabetes insipidus and prevents the formation of calcium kidney stones in people with increased levels of calcium in their urine [3, 4]. Cilnidipine (CIL) is chemically designated as 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine carboxylic acid 2-methoxy ethyl (2E)-3-phenyl-propenyl ester (Fig.1c.). It is a dual blocker of L-type voltage-gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals that supply blood vessels [5]. The literature survey reveals that all three drugs have specified developed method reported by many researchers, but with respect to double divisor ratio spectra derivative no such reports are available for these mentioned drugs [6-23].

Theoretical background [24]

This is new method for simultaneous determination of the three compounds in ternary mixtures without need to know the ratio of concentration of species. This method is based on the successive derivative of ratio spectra in two successive steps.

Consider a mixture of three compounds X, Y and Z. If Beer's law is obeyed in the whole wavelength range used and by considering the path length as 1 cm, the absorbance of the ternary mixture at each wavelength can be written as:

$$A\text{m} = \alpha\text{X}\ C\text{X} + \alpha\text{Y}\ C\text{Y} + \alpha\text{Z}\ C\text{Z}\ldots\ldots\ldots(1)$$

where Am is the vector of the absorbance of the mixture, αX ,αY and αZ are the absorptivity vectors of X, Y and Z and CX , CY and CZ are the concentrations of X, Y and Z, respectively.

If Eq. (1) is divided by αZ corresponding to the spectrum of a standard solution of Z in ternary mixture, the first ratio spectrum is obtained in the form of Eq. (2) (for possibility of dividing operation, the zero values of αZ should not be used in the divisor)

$$B = \frac{Am}{\alpha Z} = \frac{\alpha XCX}{\alpha Z} + \frac{\alpha YCY}{\alpha Z} + CZ \ldots\ldots\ldots(2)$$

If the first derivative of Eq. (2) is taken, since the derivative of a constant (CZ) is zero, Eq. (3) would be obtained:

$$\frac{dB}{d\lambda} = \frac{d}{d\lambda} = \left[\frac{\alpha XCX}{\alpha Z}\right] + \frac{d}{d\lambda}\left[\frac{\alpha YCY}{\alpha Z}\right] \ldots\ldots\ldots(3)$$

By dividing Eq. (3) by (d/dλ) (αY /αZ), corresponding to the derivative of the ratio of the spectra of the standard solutions of Y and Z, the second ratio spectrum is obtained as Eq. (4) (for possibility of dividing operation, the zero values of (d/dλ)(αY /αZ) should not be used in the divisor):

$$D = \frac{dB/d\lambda}{(d/d\lambda)(\alpha Y/\alpha Z)} = \frac{(d/d\lambda)\left[\alpha XCX/\alpha Z\right]}{(d/d\lambda)(\alpha Y/\alpha Z)} + CY \ldots\ldots\ldots(4)$$

If the first derivative of Eq. (4) is taken since the derivative of a constant (CY) is zero, Eq. (5) would be obtained

$$\frac{dD}{d\lambda} = \frac{d}{d\lambda}\left[\frac{(d/d\lambda)\left[\alpha XCX/\alpha Z\right]}{(d/d\lambda)(\alpha Y/\alpha Z)}\right] \ldots\ldots\ldots(5)$$

Eq. (5) is the mathematical foundation of multi-component analysis that permits the determination of concentration of each of the active compounds in the solution (X in this equation) without interference from the other compounds of the ternary system (Y and Z in these equations).

As Eq. (5) shows there is a linear relation between the amount of dD/dλ and the concentration of X in the solution.

A calibration curve could be constructed by plotting dD/dλ against concentration of X in the standard solutions of X or in the standard ternary mixtures.

For more sensitivity the amount of dD/dλ corresponding to maximum or minimum wavelength should be measured. Calibration graphs for Y and Z could be also constructed as described for X.

MATERIALS AND METHODS

TEL, CHL and CIL active pharmaceutical ingredients were obtained as a gift sample from Alembic research center, India. Commercial formulation (CILACAR TC 12.5 Tablet, J.B.Chemicals and pharmaceuticals LTD, Daman) containing TEL (40 mg), CHL (12.5 mg) and CIL (10 mg) were used for study. All the chemicals used were of analytical grade (Merck private limited, India).

Instrumentation and conditions

Spectral scans were analyzed on a Shimadzu UV spectrophotometer, model 1800 (Schimadzu, Japan) with automatic wavelength corrections using a pair of 10 mm quartz cells. All Spectral measurements were done using UV-Probe 2.35 software.

Preparation of standard stock and calibration solution

Accurately weighed 10 mg TEL, CHL and CIL were dissolved and diluted with methanol up to 10 ml, separately (1000 µg/mL). Further 5 ml of the above solution diluted to 50 ml with methanol to obtain a working standard solution having a concentration of 100 µg/ml. Appropriate aliquots were diluted up to

10 ml with methanol to prepare final concentration in the range of 10-50 µg/ml for TEL, 7.5-17.5 µg/ml for CHL and 5-25 µg/ml for CIL.

Preparation of sample solutions

Marketed formulation was used in analysis. Accurately weighed 20 tablets were powdered finely and weighed 444.80 mg of powder equivalent to 40 mg of TEL, 25.5 mg of CHL and 10 mg of CIL and transferred into 100 ml volumetric flask and added 30ml methanol was added and the solution was ultrasonicated for 10 min. The volume was made up to the mark with methanol and again ultrasonicated for 10 min. The solution was filtered using whatman paper 0.45 µm. 1 ml of the diluted drug solution was taken in 10 ml volumetric flask and the volume was made up with methanol.The resultant sample was used for the assay.

Validation of spectrophotometric method [25]:

Accuracy: Accuracy was determined by calculating recovery of TEL, CHL and CIL by the standard addition method. The Known amounts of standard solutions of TEL, CHL and CIL were added to the re-quantified test solutions. Each solution was measured in triplicate, and the recovery was calculated by measuring amplitude.

Precision: The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples.

Repeatability: Standard solutions of TEL, CHL and CIL were prepared of linearity range and spectrums were recorded. Amplitudes were measured at 262.40 nm, 270 nm and 251.41 nm respectively.

Intra and inter day precision: in the results within the same day (intraday), variation of results between days (interday) was analyzed. Intraday precision was determined by analyzing TEL, CHL and CIL individually three times in the same day at 262.40 nm, 270 nm and 251.41 nm respectively. Inter day precision was determined by analyzing TEL, CHL and CIL individually daily for three days at 262.40 nm, 270 nm and 251.41nm respectively.

Linearity and Range: The linearity of the analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in the sample within a given range. The range of the analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy, and linearity.

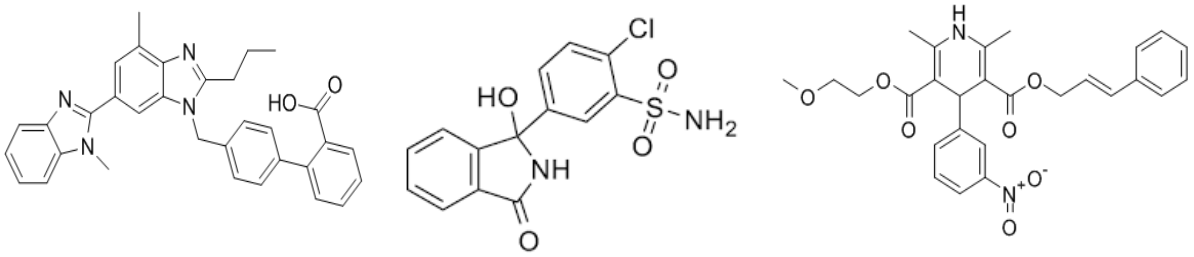
RESULTS AND DISCUSSION

Selection of analytical wavelength

Zero-order absorption (D^0) overlay spectra of TEL, CHL, and CIL revealing that their simultaneous determination is difficult in their combined dosage form by simultaneous equation spectrophotometric method is shown in Fig. 2. In context to this, as described above, the successive ratio derivative spectra method was proposed. The chosen divisor concentration gave good results for the slope, intercept and correlation coefficient of calibration graphs. The acceptable correlation coefficient was obtained for the wavelength selected for estimation of TEL (262.40 nm), CHL (270 nm) and CIL (262.40 nm) using method respectively are shown in the Fig. 3-5.

Method validation

Method validation was performed according to the ICH guideline for proposed methods [25]. The Linearity of the proposed methods was evaluated and good linearity is evident from the high value of the correlation coefficient (Table 1). The limit of detection and limit of quantification of the developed method shows the high sensitivity of the method (Table 1).The Precision of the proposed method in terms of repeatability and intermediate precision was evaluated at three concentrations of the calibration curve and percentage RSD was found to be less than 2 indicating reproducibility of both the developed method (Table 2). Accuracy further assessed by the standard addition method at three concentration levels in tablet formulation showed a mean percentage recovery at all three levels in the range suggesting the suitability of the method to perform routine drug analysis (Table 3). Percentage amount found in all the three drugs from marketed formulation was within the range 99.0400 % to 101.1924 % for method revealing no interference from the excipients and good accuracy of the proposed methods (Table 4).



(a) Telmisartan (b) Chlorthalidone (c) Cilnidipine

Fig. 1. Chemical structure of (a) Telmisartan, (b) Chlorthalidone and (c) Cilnidipine

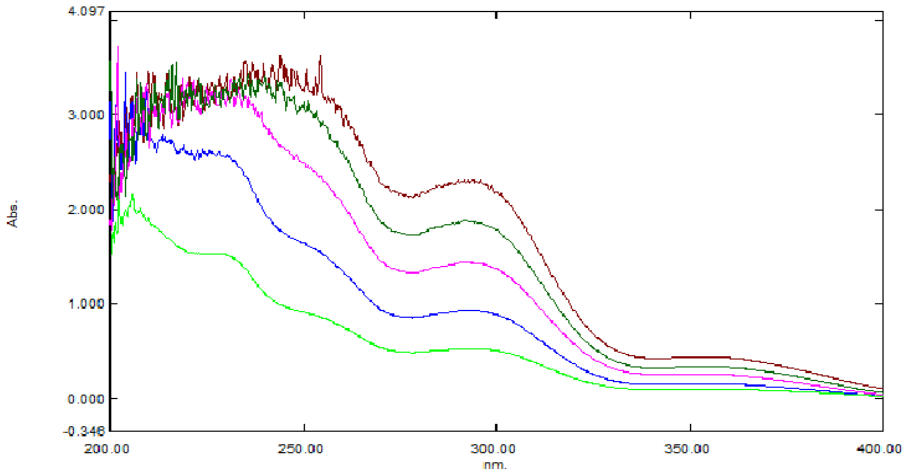


Fig. 2. Zero-order (D^0) absorption spectrum of TEL, CHL and CIL mixture solution

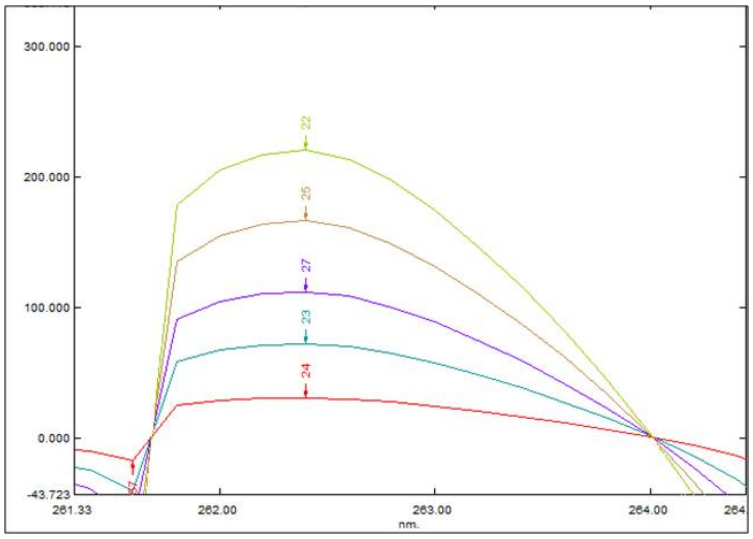


Fig. 3. Successive ratio derivative spectrum of Telmisartan (10-50 µg/ml) at 262.40 nm

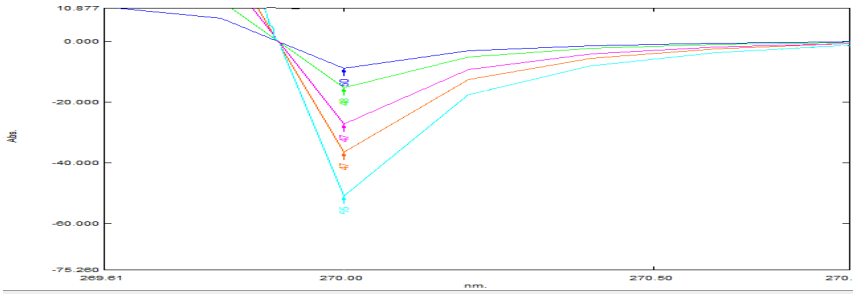


Fig. 4. Successive ratio derivative spectrum of CHLO(7.5-17.5 µg/ml) at 270 nm

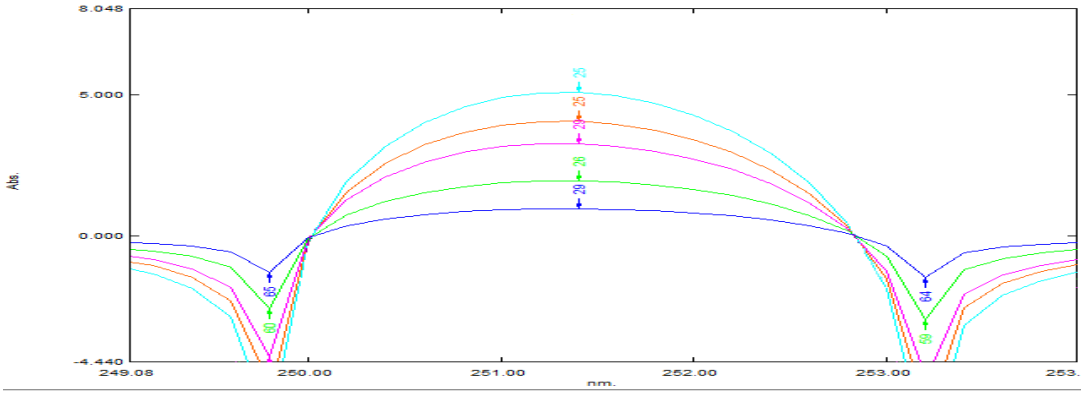


Fig. 5. Successive ratio derivative spectrum of CIL (5-25 µg/ml) at 251.41 nm

Table 1: Linear regression parameters of TEL, CHL and CIL

Parameters	TEL	CHL	CIL
Linearity range (µg/mL)	10 to 50	7.5 to 17.5	5 to 25
Regression equation	y = 0.0782x – 22.008	y = 4.4327x – 26.433	y = 0.2068x -0.0852
Correlation coefficient (r^2)	0.9952	0.9927	0.9981
Slope	0.0782	4.4327	0.2068
Intercept	22.008	26.433	0.0852
Limit of detection (µg/mL)	0.3203	1.2540	0.6024
Limit of quantitation (µg/mL)	0.9708	3.8000	1.8250

Table 2: Precision study for TEL, CHL and CIL by proposed method

Conc. (µg/ml)	Repeatability	Intermediate precision	
		Day 1	Day 2
	Amplitude[a]± %RSD (µg/ml)	Amplitude[a]± %RSD (µg/ml)	Amplitude[a]± %RSD (µg/ml)
TEL			
10	30.0456 ± 0.0013	30.714 ± 0.0381	30.3793 ± 0.0202
30	112.5526 ± 0.0011	113.5606 ± 0.0154	112.236 ± 0.0043
50	220.0813 ± 0.0015	221.0886 ± 0.0058	221.089 ± 0.0015
CHL			
7.5	7.9406 ± 0.0046	8.222 ± 0.0610	8.3133 ± 0.0705
12.5	27.8803 ± 0.0004	28.16 ± 0.0145	28.25 ± 0.0168
17.5	50.9653 ± 0.0002	51.2766 ± 0.0114	51.2696 ± 0.0054
CIL			
5	0.9973 ± 0.0050	0.9993 ± 0.0045	1.00033 ± 0.0051
15	2.8916 ± 0.0005	2.9000 ± 0.0029	2.8056 ± 0.0483
25	5.1193 ± 0.0017	5.1433 ± 0.0022	5.1616 ± 0.0020

a = mean of three determinations at three concentration level of standard; RSD=relative standard deviation

Table 3: Recovery study at three concentration levels for TEL, CHL and CIL by proposed method

% Spike Level	Amt of test taken (µg/mL)	Amt of drug added (µg/mL)	Total amount of drug taken	% Mean Recovery	SD	%RSD
TEL						
50	10	20	30	99.16666	1.3419	0.0135
100	20	20	40	98.625	0.4259	0.0043
150	30	20	50	100.14	0.6293	0.0062
CHL						
50	3.12	6.25	9.37	99.35	0.2233	0.002248
100	6.25	6.25	12.5	101.15	2.7215	0.0268
150	9.37	6.25	15.62	99.8	0.0650	0.000652
CIL						
50	2.5	5	7.5	98.03	0.7505	0.1012
100	5	5	10	99.53	0.5507	0.0551
150	7.5	5	12.5	98.28	1.6488	0.1375

[a]n= 3 replicates, S.D=standard deviation, %RSD=relative standard deviation

Table 4: Analysis of TEL, CHL and CIL in the marketed formulation by proposed method

Brand name	Drugs	Label claim (mg)	% Mean Recovery [a]	%RSD
CLICAR® TC	TEL	40	101.1924	0.7543
	CHL	12.5	99.1896	0.9861
	CIL	10	99.0400	1.2317

[a]= mean=3 replicate

CONCLUSION

A novel and simple successive ratio derivative spectra method was developed for the determination of TEL, CHL and CIL in ternary mixture and tablet formulation using methanol as a solvent without any prior separation. The validation of proposed methods according to ICH guideline proved that the method is simple, precise, reliable and accurate. These validated methos showed good recovery for all the three drugs and hence can be used in routine quality control for simultaneous estimation of the mentioned drugs in ternary mixture and pharmaceutical formulation.

CONFLICT OF INTEREST

The authors declare they have no competing interest.

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