Indo Global Journal of Pharmaceutical Sciences, 2021; 11(1): 1-6

INDO GLOBAL JOURNAL OF PHARMACEUTICAL SCIENCES
ISSN 2249- 1023

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

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**Received:** 29.06.2019
**Accepted:** 03.02.2020
**Published:** 30.03.2021
**Keywords** Telmisartan (TEL), Chlorthalidone (CHL), Cilnidipine (CIL) and Double divisor ratio derivative spectra method.

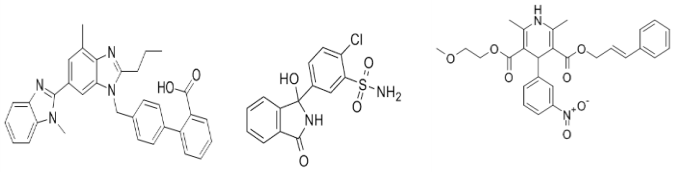
**ABSTRACT:** Aim: Development and Validation of Double Divisor Ratio Spectra Derivative Method for Simultaneous Estimation of Telmisartan, Chlorthalidone and Cilnidipine in Tablet Dosage Form. Method: A new method was developed for the simultaneous quantification of ternary mixtures by double divisor spectra method and validated as per International Conference on Harmonization [(ICH) Q2 (R1)] guideline. In double divisor spectra method absorption spectrum of ternary mixture divided by two of the three compounds in the mixture. 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. Results: 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. Conclusion: The proposed method demonstrated that the method is accurate, simple, precise, compounds not interference with each other and no any prior separation of compounds. 

**Cite this article as:** Patel, D.; Patel, P.; Patel, J. Development and validation of double divisor ratio spectra derivative method for simultaneous estimation of telmisartan, chlorthalidone and cilnidipine in tablet dosage form. Indo Global J. Pharm. Sci., 2021; 11(1): 1-6. **DOI**: http://doi.org/10.35652/IGJPS.2021.111001 .

## 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].



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

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

The literature survey reveals that all three drugs have specific 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 method is based on the use of the derivative of the ratio spectrum obtained by dividing the absorption spectrum of the ternary mixture by a standard spectrum of a mixture of two of the three compounds in the mixture, and the measuring at either the maximum or minimum wavelengths. It can only be used for mixtures that the ratio of the concentrations of two onther interfering compounds (used as double divisor) is known. In other words, the ratio of the concentrations of two interfering compounds should be the same in calibration, prediction and unknown samples. It is obvious that the ratio of the concentration of the analytes in real samples is always unknown.

If a mixture of three compounds (x, y and z) is considered, if the Beer's law is obeyed for all compounds over the whole wavelength range used and if the path length is 1 cm, the absorption spectrum of the ternary mixture at wavelength λ can be written in the form of the equation

$$A_m = a_x c_x + a_y c_y + a_z c_z \quad (1)$$

Where $A_m$ is the absorbance of the mixture, $a_x$, $a_y$, and $a_z$ are the absorptivity of x, y and z, and

$c_x$, $c_y$ and $c_z$ are the concentrations of x, y and z, respectively.

A similar equation for two compounds in the same ternary mixture as in a standard binary mixture can be written as

$$Am = ax\ cx + ay\ cy \quad (2)$$

If Eq. (1) is divided by Eq. (2) corresponding to the spectrum of a standard solution of two of the components in the ternary mixture, the ratio spectrum is obtained in the form of Eq. (3)

$$A_m/ a_x c_x + a_y c_y = a_x c_x + a_y c_y/ a_x c_x + a_y c_y + a_z c_z/ a_x c_x + a_y c_y \quad (3)$$

The ratio of the sum of ($a_x c_x + a_y c_y$) to the sum of ( $a_x c_x + a_y c_y$ ) is equal to a constant (k) with respect to λ. If the above constant is replaced in Eq. (3), we obtain Eq. (4)

$$A_m/ a_x c_x + a_y c_y = k\ (\text{constant}) + a_z c_z/ a_x c_x + a_y c_y \quad (4)$$

However, if the standard concentrations of cx and cy in Eq. (2) are equal or very close to each other, cx = cy or cx = cy, we could write

$$a_x c_x + a_y c_y = cx\ (ax + ay) \quad (5)$$

When Eq. (5) is substituted into Eq. (4), Eq. (6) is obtained

$$A_m/ c_x (a_x + a_y) = k\ (\text{constant}) + a_z c_z/ c_x (a_x + a_y) \quad (6)$$

If the first derivative of Eq. (6) is taken, since the derivative of a constant is zero, Eq. (7) would be obtained

$$d/d\lambda\ [A_m/ c_x (a_x + a_y)] = d/d\lambda\ [a_z/(a_x + a_y)]\ c_z/c_x \quad (7)$$

Eq. (7) is the mathematical foundation of multicomponent analysis, which permits the determination of the concentration of each of the active compounds in solution without interference from the other components of the ternary system. In practice, Eq. (7) corresponding to the first derivative ratio spectrum of z is obtained by dividing the absorption spectrum of the ternary mixture of x, y and z by the standard spectrum of two of the compounds in the ternary mixture. Also, in Eq. (7), the derivative signal of the ratio spectrum of the ternary mixture is dependent only on the concentration values $C_Z$ and $c_x$, but is independent of the concentration values $C_X$ and $C_y$ in the ternary mixture. The concentrations of x and y are determined by analogous procedures.

# 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 weighted 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.

**Double divisor ratio derivative spectra method**

For determination of TEL, the stored spectra of the solutions of a ternary mixture were divided by the sum of the standard spectrum of CIL (20 µg/ml) and CHL (14 µg/ml) to record ratio spectra, Followed by the first derivative transformation of these vectors with respect to wavelength. The minimum or maximum of the second derivative of these ratio spectra with respect to wavelength was recorded for the construction of the calibration curve. A Similar procedure was followed for the other two components; CHL and CIL by using a divisor concentration of TEL (50 µg/ml) + CIL (17.5 µg/ml) and TEL (40 µg/ml) + CHL (14 µg/ml) respectively.

**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 316.31 nm, 390.59 nm and 337.39 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 316.31 nm, 390.59 nm and 337.39 nm respectively. Inter day precision was determined by analyzing TEL, CHL and CIL individually daily for three days at 316.31 nm, 390.59 nm and 337.39 nm 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. In context to this, as described above, the double divisor 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 (316.31 nm), CHL (390.59 nm) and CIL (337.39 nm) using method respectively are shown in the **Fig. 3 – 5**.

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

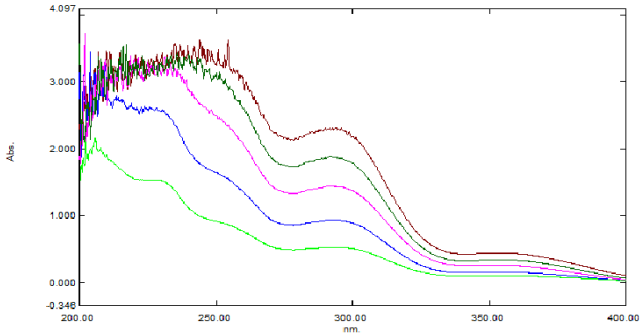



**Fig. 3. First order derivative ($D^1$) ratio spectra of TEL 316.31 nm**

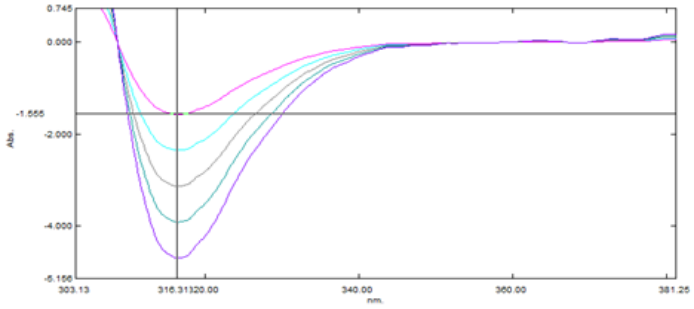


**Fig. 4. First order derivative ($D^1$) ratio spectra of CHL 390.59 nm**

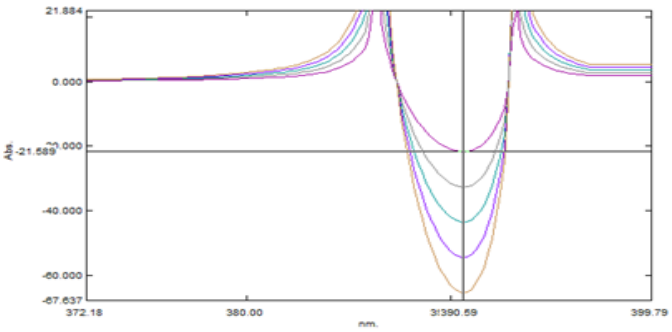
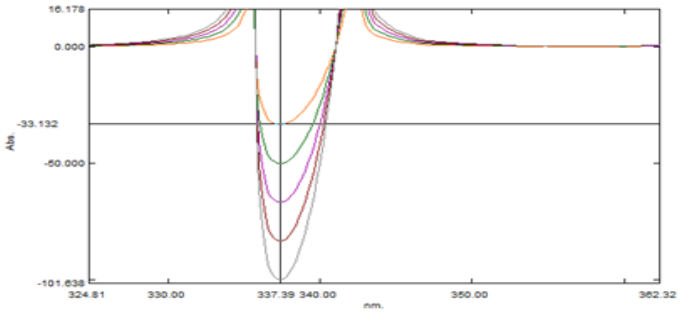



**Fig. 5. First order derivative ($D^1$) ratio spectra of CIL 337.39 nm**




| Method parameters | Optimized parameters |
|---|---|
| Solvent | Methanol |
| Scanning range | 200-400 nm |
| Scan speed | Medium |
| Divisor conc. for determination of TEL | CIL (20 µg/ml) + CHL (14 µg/ml) |
| Divisor conc. for determination of CHL | TEL (50 µg/ml) + CIL (17.5 µg/ml) |
| Divisor conc. for determination of CIL | TEL (40 µg/ml) + CHL (14 µg/ml) |
| Analytical wavelength for determination of TEL | 316.31 nm |
| Analytical wavelength for determination of CHL | 390.59 nm |
| Analytical wavelength for determination of CIL | 337.39 nm |

**Table 1. Optimized method parameters for ratio derivative spectrophotometry.**

**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 2**). The limit of detection and limit of quantification of the developed method shows the high sensitivity of the method (**Table 2**). 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 3**). 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 of 99.3425 % to 100.9414 %, suggesting the suitability of the method to perform routine drug analysis (**Table 4**). 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 5**).

| Parameters | TEL | CHL | CIL |
|---|---|---|---|
| Linearity range (µg/mL) | 10 to 50 | 7.5 to 17.5 | 5 to 25 |
| Regression equation | y = 0.0782x + 0.8055 | y = 3.2491x – 17.7930 | y = 4.5264x -11.9121 |
| Correlation coefficient ($r^2$) | 0.9956 | 0.9982 | 0.9969 |
| Slope | 0.8055 | 3.2491 | 4.5264 |
| Intercept | 0.8055 | 17.7930 | 11.9121 |
| Limit of detection (µg/mL) | 0.3203 | 1.2540 | 0.6024 |
| Limit of quantitation (µg/mL) | 0.9708 | 3.8000 | 1.8250 |

**Table 2: Linear regression parameters of TEL, CHL and CIL.**

| 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** | 1.552 ± 0.0222 | 1.612 ± 0.0208 | 1.624 ± 0.0226 |
| **30** | 3.214 ± 0.0173 | 3.468 ± 0.0195 | 3.384 ± 0.0190 |
| **50** | 4.667 ± 0.0136 | 4.847 ± 0.0114 | 4.692 ± 0.0105 |
| **CHL** | | | |
| **7.5** | 21.589 ± 0.0113 | 22.538 ± 0.0148 | 22.281 ± 0.0187 |
| **12.5** | 43.326 ± 0.0060 | 42.386 ± 0.0079 | 42.273 ± 0.0060 |
| **17.5** | 65.162 ± 0.0056 | 64.317 ± 0.0054 | 64.538 ± 0.0049 |
| **CIL** | | | |
| **5** | 33.312 ± 0.0222 | 33.187 ± 0.0208 | 34.816 ± 0.0226 |
| **15** | 66.632 ± 0.0173 | 67.495 ± 0.0195 | 68.591 ± 0.0190 |
| **25** | 98.856 ± 0.0136 | 98.381 ± 0.0114 | 95.361 ± 0.0105 |

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

**Table 3: Precision study 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 | 100.6938 | 0.1477 | 0.1466 |
| **100** | 20 | 20 | 40 | 99.7711 | 0.8386 | 0.8406 |
| **150** | 30 | 20 | 50 | 100.9414 | 0.1457 | 0.1443 |
| **CHL** | | | | | | |
| **50** | 3.12 | 6.25 | 9.37 | 99.3425 | 0.3517 | 0.3540 |
| **100** | 6.25 | 6.25 | 12.5 | 99.5666 | 0.6765 | 0.6794 |
| **150** | 9.37 | 6.25 | 15.62 | 100.0681 | 0.4562 | 0.4559 |
| **CIL** | | | | | | |
| **50** | 2.5 | 5 | 7.5 | 99.6320 | 0.1309 | 0.1314 |
| **100** | 5 | 5 | 10 | 100.0700 | 0.0578 | 0.0570 |
| **150** | 7.5 | 5 | 12.5 | 99.9454 | 0.1124 | 0.1121 |

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

**Table 4: Recovery study at three concentration levels for TEL, CHL and CIL 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 replicates

**Table 5: Analysis of TEL, CHL and CIL in the marketed formulation by proposed method.**

# CONCLUSION

A novel and simple double divisor 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 methods 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.

# CONFLICTS OF INTEREST

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

# ACKNOWLEDGEMENT

None

# DATA AVAILABILITY

Not declared.

## FUNDING SOURCE

Not declared

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*Indo Global Journal of Pharmaceutical Sciences( ISSN 2249 1023; CODEN- IGJPAI; NLM ID: 101610675) indexed and abstracted in CrossRef (DOI Enabling), CNKI, EMBASE (Elsevier), National Library of Medicine (NLM) Catalog (NCBI), ResearchGate, Publons (Clarivate Analytics), CAS (ACS), Index Copernicus, Google Scholar and many more. For further details, visit http://iglobaljournal.com*