Vierodt/Simultaneous Equation Method

Procedure:

Step 1: Preparation of standard solution of Telmisartan

i. Standard stock solution of Telmisartan is prepared by dissolving 10 mg of Telmisartan drug in a 10 mL volumetric flask using methanol (If drug is not dissolved, sonicate it for 15 minutes). Stock solutions of 1000 µg/mL were obtained in this manner.
ii. Then, take pipette out 1 ml of solution from above prepared 1000 µg solution and add it into 10 ml volumetric flask. Make up the volume up to the mark with pH 7.5 phosphate buffer as a solvent. A solution of 100 µg is prepared.
iii. From these 100 µg stock solutions, working standard solutions of concentration 5, 10, 15, 20, 25 µg/mL each were prepared by appropriate dilutions.
iv. Working standard solutions of concentration 10 µg/mL were scanned in the entire UV range to determine the λmax. Use solution prepared by dissolving (1 ml methanol + 9 ml pH 7.5 phosphate buffer) as a blank solution.
v. In our case the λmax of Telmisartan were found to be 295 nm.

Step 2: Preparation of standard solution of Hydrochlorothiazide

i. Standard stock solution of Hydrochlorothiazide is prepared by dissolving 10 mg of Hydrochlorothiazide drug in a 10 mL volumetric flask using methanol (If drug is not dissolved, sonicate it for 15 minutes). Stock solutions of 1000 µg/mL were obtained in this manner.
ii. Then, take pipette out 1 ml of solution from above prepared 1000 µg solution and add it into 10 ml volumetric flask. Make up the volume up to the mark with 0.1 M HCl solution as a solvent. A solution of 100 µg is prepared.
iii. From these 100 µg stock solutions, working standard solutions of concentration 5, 10, 15, 20, 25 µg/mL each were prepared by appropriate dilutions.
iv. Working standard solutions of concentration 10 µg/mL were scanned in the entire UV range to determine the λmax. Use solution prepared by dissolving (1 ml methanol + 9 ml 0.1 M HCl) as a blank solution.
v. In our case the λmax of Hydrochlorothiazide were found to be 224 nm.

Step 3: Take absorbance of all stock solution of Telmisartan

i. Measure the absorbance of all the five-stock solution of concentration 5, 10, 15, 20, 25 µg/mL of Telmisartan on UV at both wavelength of 295 nm and 224 nm. Use solution prepared by dissolving (1 ml methanol + 9 ml pH 7.5 phosphate buffer) as a blank solution.

ii. Note the absorbance of all the stock solutions of Telmisartan in below table.

Table 1: Absorbance of stock solutions of Telmisartan		
Conc.	**Absorbance at λ1 = 295 nm**	**Absorbance at λ2 = 224 nm**
5 µg/mL	0.221	0.416
10 µg/mL	0.332	0.731
15 µg/mL	0.635	1.152
20 µg/mL	0.831	1.567
25 µg/mL	1.013	1.895

Step 4: Take absorbance of all stock solution of Hydrochlorothiazide

i. Measure the absorbance of all the five-stock solution of concentration 5, 10, 15, 20, 25 µg/mL of Hydrochlorothiazide on UV at both wavelength of 295 nm and 224 nm. Use solution prepared by dissolving (1 ml methanol + 9 ml 0.1 M HCl) as a blank solution.

ii. Note the absorbance of all the stock solutions of Hydrochlorothiazide in below table.

Table 2: Absorbance of stock solutions of Hydrochlorothiazide		
Conc.	**Absorbance at λ1 = 295 nm**	**Absorbance at λ2 = 224 nm**
5 µg/mL	0.055	0.453
10 µg/mL	0.06	0.55
15 µg/mL	0.119	0.845
20 µg/mL	0.177	1.131
25 µg/mL	0.226	1.539

Step 5: Calculate the absorptivity

i. Convert the concentration of stock solution from µg/mL to µg/100 mL. Then convert it from µg/100 mL to g/100 ml.

For example,

10 µg/mL ⟶ 1000 µg/100 mL ⟶ 0.001 g/ 100 mL

ii. Then calculate the absorptivity from the absorbance by using the below formula. (Use molar concentration in gram to calculate the absorptivity)

$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M
l	optical path length	cm

For example,

Absorptivity = Absorbance/Concentration

= 0.332/0.001

= 332

Table 3: Absorptivity (Telmisartan)		
Conc.	**Absorptivity at λ1 = 295 nm**	**Absorptivity at λ2 = 224 nm**
5 µg/mL	44.2	83.2
10 µg/mL	332	731
15 µg/mL	423.3333333	768
20 µg/mL	415.5	783.5
25 µg/mL	405.2	758

Table 4: Absorptivity (Hydrochlorothiazide)		
Conc.	**Absorptivity at λ1 = 295 nm**	**Absorptivity at λ2 = 224 nm**
5 µg/mL	11	90.6
10 µg/mL	60	550
15 µg/mL	79.33333333	563.3333333
20 µg/mL	88.5	565.5
25 µg/mL	90.4	615.6

Step 6: Take the absorbance of marketed tablet

i. Purchase a tablet from market that contain the same amount of drug as of your formulated tablet. (For example, your formulated tablet contains 40 mg Telmisartan and 12.5 mg Hydrochlorothiazide then purchase a marketed tablet that should also contain 40 mg Telmisartan and 12.5 mg Hydrochlorothiazide)

ii. The marketed tablet is then crushed to a fine powder. An accurately weighed powder sample equivalent to 25 mg of Telmisartan was transferred to a 25 ml volumetric flask.

iii. Calculate the equivalent weight by using below formula.

$$Equivalent\ Weight = \frac{Quantity\ Required\ \times Average\ Weight\ of\ Tablet}{Label\ Claim}$$

iv. Then volume was made up to the mark with methanol solution and sonicate for 15 minutes to ensure the powder dissolves properly. The concentration of prepared solution will be of 1000 µg/mL.

v. The solution was then filtered through Whatman filter paper no. 41.

vi. Then pipette out 1 ml from filtered solution and make volume with pH 7.5 phosphate buffer solution to produce a stock solution of concentration 100 µg/mL.

vii. Again, pipette out 2 ml from above solution and volume was make up with pH 7.5 phosphate buffer solution to prepare a stock solution of concentration 20 µg/mL.

viii. Measure the absorbance of stock solution of concentration 20 µg/mL on UV at both wavelength of 295 nm and 224 nm. Use solution prepared by dissolving (1 ml methanol + 9 ml pH 7.5 phosphate buffer) as a blank solution.

ix. Note the readings in below table.

Table 5: Absorbance of stock solutions of marketed tablet

Conc.	Absorbance at λ1 = 295 nm	Absorbance at λ2 = 224 nm
20 µg/mL	0.895	1.871

Step 7: Determine the ax1, ax2, ay1 and ay2

i. Determine the ax1, ax2, ay1 and ay2 from the table 3 and table 4.

ii. Were,

ax1 = Absorptivity of Telmisartan at λ1

ax2 = Absorptivity of Telmisartan at λ2

ay1 = Absorptivity of Hydrochlorothiazide at λ1

ay2 = Absorptivity of Hydrochlorothiazide at λ2

Step 8: Determine the A_1 and A_2

i. Determine the A_1 and A_2 from the table 5.

ii. Were,

A_1 = Absorbance at λ1

A_2 = Absorbance at λ2

Step 9: Determine the Cx and Cy

i. Determine the Cx = Concentration of Telmisartan and Cy = Concentration of Hydrochlorothiazide by using the below formula.

$$Cx = \frac{A_2\, a_{y1} - A_1\, a_{y2}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$Cy = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

ii. The founded value of Cx will be in g/100 ml. So, convert the value in g/10 ml and then to mg/10 ml.

For example,

The founded value of Cx = 0.001986518 g/100 ml

0.000198652 g/10 ml

0.198651755 mg/10 ml

iii. It means 0.198651755 mg of Telmisartan drug is present in 2 ml of stock solution. Now find how much amount of drug should present in 10 ml by doing cross multiplication.

2	⟶	0.1986518
10	⟶	x

$$X = \frac{0.1090097 * 10}{2}$$

$$X = 0.993259$$

iv. Multiply the founded X value with 25 to obtain the drug present in 25 ml of stock solution.

$$X = 0.993259 \times 25$$

FINAL = 24.831475

v. Follow the step ii, iii and iv for obtaining the concentration of hydrochlorothiazide drug.