Method Development and Validation of simultaneous estimation for Ramipril and Amlodipine besylate by UV- VISIBLE Spectrophotometric method

Khairunnisa Tasneem, Sonia K*, Lakshmi K S

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM nagar , Kattankulathur - 603 203. Chengalpattu District, TamilNadu, India

**Article History:**
Received on: 28 Jan 2020
Revised on: 01 Mar 2020
Accepted on: 04 Mar 2020

**Keywords:**
Amlodipine besylate, method development, Ramipril, Ultra Violet and Visible spectroscopy and validation

**ABSTRACT**

This experiment shares the method development and analytical validation of an original, accurate and correct UV-Visible Spectrophotometric methods for the fixed dose assessment of Ramipril and Amlodipine besylate. The working solutions of Telmisartan and Ramipril were scanned at 240 nanometer and 210nanometer respectively. The regression strength of Amlodipine besylate and Ramipril over its absorbances take place as y=0.4291x+0.0084 and y=0.0399x-0.310 respectively with a correlation coefficient ($r^2$) of 0.9998 for Amlodipine besylate and 0.9993 for Ramipril. The intra-day precision in addition inter-day precision for Amlodipine besylate and its % RSD were obtained as 0.08% and 0.25% respectively. The intra-day precision in addition inter-day precision for Ramipril and% RSD were obtained as 0.16% and 0.24% respectively. The precise amount of tablet formulation were added which holds Alkaline (0.1 N Sodium hydroxide), Acidic (0.1 N Hydrochloric acid) reflux for 3 hours, 3% Oxydol at 50ºC, heat (60ºC), humidity (75 percentage Relative humidity) for 24 hr. and after the particular time diluted to distilled water, separated using Filter paper. From this stock solution, 5 mL section of the filtrate was pipetted out and further thinned with distilled water in a 100 mL standard flask (10 µg/mL). The standard stock solution of two drugs were prepared and compared against a label claim.

*Corresponding Author
Name: Sonia K
Phone: +91-9884328353
Email: soniapharm68@gmail.com

ISSN: 0975-7538
DOI: [https://doi.org/10.26452/ijrps.v11i3.2557](https://doi.org/10.26452/ijrps.v11i3.2557)

Production and Hosted by Pharmascope.org © 2020 | All rights reserved.

**INTRODUCTION**

Ramipril is chemically(2S,3aS,6aS)-1-[(2S)-2-[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,3a,4,5,6,6a-hexahydro-2H-cyclopenta[b]pyrrole-2-carboxylic acid. Amlodipine besylate is a long-chain acting calcium channel blocker to diagnosis hypertension and angina. (Shah et al., 2012). Chemically it is benzensulfonic acid;3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. The skeletal structure of both is given in Figure 1 & Figure 2. It precisely clogs calcium influx across cell membranes in cardiac and vascular smooth muscle, with greater strength on vascular smooth muscle (Babu et al., 2014; Jain et al., 2012). Ramipril is a prodrug and nonsulphydryl angiotensin converting enzyme (ACE) inhibitor having antihypertensive function. (Naveen et al., 2013; Maste et al., 2011). Ramipril is modified in the liver by de-esterification into its energetic manifestation ramiprilat, which restrain ACE, thereby stops the conversion of angiotensin I to angiotensin II (Patil et al., 2009; Karaaji and Kulkarni, 2013). This put to an end the potent vasoconstrictive actions of angiotensin II and leads to vasodilatation (Jam-
pana et al., 2014; Babu et al., 2014). This agent also causes an increase in bradykinin levels and a decrease in angiotensin II–induced aldosterone secretion by the adrenal cortex, thereby promoting diuresis and natriuresis (Sonia et al., 2018; Sharma et al., 2010).

MATERIALS AND METHODS

Instrumentation

Digital weighing machine from Shimadzu, Lab India Analytical UV 3092. Double beam UV–Visible Spectrophotometer, pH meter (Systronics model EQMK VI), a sonicator (Spectra Lab, model UCB 40), a hot air oven (Labhosp), UV chamber (Labhosp) were used in this study.

Materials and Reagent employed

Ramipril and Amlodipine besylate of pharmaceutical grade were supplied by Yarrow Chem Pvt Ltd. Methanol, water utilize the HPLC category in addition bought from Spectrochem Pvt. Ltd. Mumbai, India. The tablet formulation containing 5mg of Ramipril and 40mg of Amlodipine besylate was purchased from local market and used for analysis of marketed formulation.

Standard stock solution Preparation

Meticulous weighed and transferred into 50mL standard ϑlasks of 2.5milligram of each drug Amlodipine besylate and Ramipril in two separate standard ϑlasks and dissolved in 50mL of methyl alcohol sonicated for 20 minutes to get the strength of employed standard solution of 50 $\mu$g per mL of both the drugs.

Selection of Wavelength

For estimation of Ramipril employing 210 nanometer as wavelength was ϑixed. For the estimation of Amlodipine besylate 240 nanometer was selected.

Preparation of sample

Ten tablets weight were taken which is finely powdered in a mortar. The equivalent quantity of 2.5 milligram Ramipril and 5milligram of Amlodipine besylate was meticulously weighed and relocated into a 50mL tidy volumetric flask in which methyl alcohol was added and sonicated for 5min in which drug go into solution completely. The solution was separated by using whatman ϑilter paper, discarding first few ML. It is diluted till the mark by methyl alcohol to obtain stock strength of 50 $\mu$g/mL of Ramipril and 100 $\mu$g/mL of Amlodipine besylate.

RESULTS AND DISCUSSION

Method development

Amlodipine besylate and Ramipril standard compound were taken for dilution 100$\mu$g/ml. Different dilution was taken from standard stock solution

© International Journal of Research in Pharmaceutical Sciences
### Table 1: Linearity table of Amlodipine and Ramipril

| S.No. | Ramipril Conc(µg/mL) | Absorbance | Amlodipine besylate Conc(µg/mL) | Absorbance |
|-------|----------------------|------------|---------------------------------|------------|
| 1     | 10                   | 0.0767     | 0                               | 0.0287     |
| 2     | 15                   | 0.293      | 5                               | 0.2193     |
| 3     | 20                   | 0.4975     | 10                              | 0.4005     |
| 4     | 25                   | 0.6944     | 15                              | 0.6544     |
| 5     | 30                   | 0.8871     | 20                              | 0.8871     |
| 6     | 35                   | 1.0792     | 25                              | 1.0792     |
| Slope |                      | 0.0399     | Slope                           | 0.4291     |
| Intercept |                    | -0.310     | Intercept                       | 0.0084     |
| Correlation coefficient |            | 0.9993     | Correlation coefficient          | 0.997      |

### Table 2: Intra-day and Inter-day precision for Ramipril and Amlodipine besylate

| S. No. | Time (Hours) | Ramipril Absorbance | Amlodipine Absorbance | Time (Days) | Ramipril Absorbance | Amlodipine Absorbance |
|--------|--------------|---------------------|-----------------------|-------------|---------------------|-----------------------|
| 1      | 2            | 99.68               | 99.98                 | 1           | 99.75               | 99.74                 |
| 3      | 4            | 99.78               | 99.85                 | 2           | 98.98               | 99.69                 |
| 4      | 6            | 99.89               | 99.64                 | 3           | 99.69               | 99.88                 |
| 5      | 8            | 99.86               | 99.87                 | 5           | 98.79               | 99.92                 |
| 6      | 10           | 99.89               | 99.68                 | Mean        | 99.75               | 99.58                 |
| Mean   |              | 99.78               | 99.81                 | Mean        | 99.471              | 99.81                 |
| SD     |              | 0.1269              | 0.1276                | SD          | 0.4621              | 0.1322                |

### Table 3: Recovery studies for Ramipril and Amlodipine besylate

| Std. conc. (µg/mL) | Ramipril 80% | 100% | 120% | Amlodipine besylate 80% | 100% | 120% |
|--------------------|--------------|------|------|-------------------------|------|------|
| Conc. added (µg/mL)| 10           | 10   | 10   | 10                      | 10   | 10   |
| Conc. found (µg/mL)| 8            | 10   | 12   | 8                        | 10   | 12   |
| % Recovery         | 99.375       | 99.1 | 99.5 | 99.62                   | 99.8 | 99.33|
| % Mean recovery    | 99.32        |      |      |                         |      |      |

### Table 4: Stability studies parameters for Ramipril and Amlodipine besylate

| Sample (treated)      | Ramipril | Amlodipine besylate |
|-----------------------|----------|---------------------|
| 0.1 N NaOH            | 99.77    | 98.14               |
| 0.1 N HCl             | 96.21    | 96.82               |
| 60ºC for 2hr          | 99.85    | 98.01               |
| Humidity (75% RH)     | 98.97    | 97.92               |

### Table 5: LOD and LOQ for Ramipril and Amlodipine besylate

| Parameter                     | Ramipril value(µg/mL) measured | Amlodipine besylate value(µg/mL) measured |
|-------------------------------|--------------------------------|------------------------------------------|
| Limit of detection            | 0.25                           | 0.37                                     |
| Limit of quantification       | 0.34                           | 0.97                                     |

© International Journal of Research in Pharmaceutical Sciences
Table 6: Assay of Ramipril and Amlodipine besylate formulations

| Formulation          | Label claim | Amount found | % Assay |
|----------------------|-------------|--------------|---------|
| Raptakos Ramipril    | 2.5mg       | 2.4mg        | 96      |
| Amlodipine besylate  | 5mg         | 4.8mg        | 96      |

and diluted with methyl alcohol in the strength of 5µg/mL to 25 µg/mL solutions at 2µg/mL interval. The working solutions of Telmisartan and Ramipril were prepared and scanned at 240 nanometer and 210 nanometer respectively. The absorbances were recorded and are outlined against the strength to obtain the respective calibration curves.

Validation of the method

The technique has been endorsed, in accordance with ICH guidelines ICH Q2B, for linearity, Precision, Stability parameters,

Linearity

For the estimation of Amlodipine besylate and Ramipril lamda max were found to be 240 nanometer and for Ramipril was found to be 210nanometer in methyl alcohol solvent. The linearity for Amlodipine besylate and Ramipril in the strength range of 5-25 µg/mL and 10- 35 µg /mL. (Table 1 and Figure 3 and Figure 4).

Precision

For the intra-day calculation of precision 0-10 hours with the interval of every two hours and interday precision 1-6 days were chosen and readings were taken for every day for Ramipril and Amlodipine besylate and tabulated in Table 2.

Accuracy

Accuracy was determined for drugs by spiking with 80, 100 and 120 percentage of pure drug and the mean recovery of the Ramipril and Amlodipine besylate were to be 96% and 96% respectively (Table 3).

Stability parameter

The precise amount of tablet formulation which is equal to 5 milligram of Amlodipine besylate and 2.5 milligram of Ramipril was transferred into 100 mL standard flask and maintained under the subsequent conditions which holds Alkaline(0.1 N Sodium hydroxide), Acidic (0.1 N Hydrochloric acid) reflux for 3 hours, 3% Oxydol at 50ºC, heat (60ºC), humidity (75 percentage Relative humidity) for 24 hr. and after the particular time diluted to distilled water, separated using Filter paper. From this stock solution, 5 mL section of the filtrate was pipetted out and further thinned with distilled water in a 100 mL standard flask (10 µg/mL). The standard stock solution of two drugs were prepared and compared against a label claim and results were tabulated in Table 4.

Detection limit and quantification limit

The detection limit (LOD) and quantification limit (LOQ) for Ramipril verified to be 0.25µg/mL and 0.34µg/mL respectively. The detection limits (LOD) and quantification for Amlodipine besylate designate as 0.37µg/mL and 0.94 µg/mL (Table 5) respectively.

Assay

The assay of Ramipril and Amlodipine besylate were done and its percentage purity designate as 99.60% and 100.04 % respectively (Table 6).

CONCLUSIONS

The extent of Amlodipine besylate another Ramipril bulk samples and their tablet forms were determined by simultaneous equation method by using UV Spectrophotometer. The regression strength of Amlodipine besylateand Ramipril over its absorbances were obtained as y=0.4291x0.0084 and y=0.0399x-0.310 respectively with a correlation coefficient ($r^2$) of 0.9998 for Amlodipine besylateand 0.9993 for Ramipril. The intra-day precision in additionto inter-day precision for Amlodipine besylate and its % RSD were obtained as 0.08% and 0.25% respectively. The intra-day precision in addition to inter-day precision for Ramipril and% RSD were obtained as 0.16% and 0.24% respectively. This confirms the procedure is precise. Accuracy is determined for both drugs by spiking with 80, 100 and 120% of additional pure remedy and the % mean recovery of the Amlodipine besylateand Ramipril were obtained as 99.58 and 99.32 respectively. The percentage purity for the assay of Amlodipine besylateand Ramipril were obtained as 96% and 96% respectively. The assay result shows that the methodology was selective for evaluation of Amlodipine besylateand Ramipril without hindering from the inactive substance used in tablet dosage form.

Conflict of Interest

None.

Funding Support

© International Journal of Research in Pharmaceutical Sciences
REFERENCES

Babu, R. G., Kumar, P., Surekha, J. S. L., Praveen, P. K., T, Rao, S. 2014. Development and Validation of UV Spectrophotometric Method of Amlodipine Besylate in Bulk and Pharmaceutical Formulation. Asian Journal of Research in Chemistry, 7(6):2014–2021.

Jain, P. S., Patel, M. K., Bari, S. B., Surana, S. J. 2012. Development and validation of HPTLC method for simultaneous determination of amlodipine besylate and metoprolol succinate in bulk and tablets. Indian Journal of Pharmaceutical Sciences, 74(2):152–152.

Jampana, P., Kumar, R., Varma, B. H., Babu, R. G., Surekha, J. P. S. L., T, K. P., R, S. 2014. Visible Spectroscopic method for estimation of Amlodipine besylate in Tablets. International Journal of Pharmaceutical Chemical & Biological Sciences, 4(1):173–177.

Karajgi, S., Kulkarni, R. 2013. Simultaneous estimation of Ramipril and Olmesartan Medoxomil by first derivative UV Spectrophotometric method. Annalen Der Chemischen Forschung, 1(3):26–32.

Maste, M. M., Kalekar, M. C., Kadian, N., R, B. A. 2011. Development of RP-HPLC Method for Simultaneous Estimation of Amlodipine and Ramipril in Tablet Dosage form. Asian Journal of Research Chemistry, 4(8):1210–1213.

Naveen, B., Ganesh, T., Kavitha, B., Reddy, G. N. 2013. Development and Validation of Ramipril and Amlodipine by RP-HPLC method in bulk and pharmaceutical dosage form. International Journal of Chemical and Natural Science, 1(1):17–20.

Patil, P., Rakesh, S., Dhabale, P., Burade, K. 2009. Simultaneous Estimation of Ramipril and Amlodipine by UV Spectrophotometric Method. Research Journal of Pharmacy and Technology, 2(2):304–307.

Shah, S., Asnani, A. J., Kawade, D., Dangre, S. C. 2012. Spectrophotometric Method for Simultaneous Estimation of Olmesartan Medoxomil and Amlodipine Besylate in Pharmaceutical Preparations. Research J. Pharm. and Tech, 5:955–957.

Sharma, T., Mishra, N., Si, C., Shankar, S., D 2010. Simultaneous estimation of Olmesartan medoxomil and amlodipine besylate in tablet dosage form. Der Pharmacia Lettre, 2:302–307.

Sonia, Manikandan, K., Ndwabe, H., Sree, P. B., Lakshmi, K. S. 2018. Method Development and Validation of simultaneous estimation for Amlodipine besylate and Olmesartan medoxomil by HPTLC method. International Journal of Research in Pharmaceutical Sciences, 9(1):201–201.