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Simultaneous Estimation of Amlodipine Besylate and Ramipril in Tablets Dosage Form by UV Spectrophotometric Method.

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Abstract

Amlodipine Besylate is used to treat hypertension and Coronary Artery Disease. It is a calcium channel blocker. Amlodipine Besylate blocks calcium movement into certain tissues and arteries which leads to the relaxation of arteries so that blood can flow more easily into our heart. Ramipril is also used to treat hypertension, prevent strokes, heart attacks and kidney problems. It is an angiotensin converting enzyme (ACE) inhibitors. ACE is an enzyme which produces the chemical angiotensin II. No or only a few spectrophotometric methods have been reported for simultaneous estimation of Amlodipine Besylate and Ramipril in tablet dosage forms. Hence an attempt has been made to develop and validate in accordance with ICH guidelines.

Keywords: Amlodipine Besylate, Ramipril, UV Spectroscopy and Simultaneous Equation Method.

INTRODUCTION

Amlodipine Besylate or 3-ethyl 5-methyl 2-[(2- aminoethoxy) methyl]-4-(2-chlorophenyl)-6--methyl-1, 4-dihydropyridine-3, 5-dicarboxylate (Figure 1) is used to treat hypertension and coronary artery disease [1,2 and 3]. It is a calcium channel blocker. Amlodipine blocks calcium movement into certain tissues and arteries which leads into relaxation of arteries so that blood can flow more easily into our heart [7, 10 and 15]. It also reduces chances of heart attack or stroke. The most common side effects of Amlodipine are: - swelling of your legs or ankles, tiredness, stomach pain, nausea, dizziness [6, 16 and 17].

Ramipril or [(2S, 3aS, 6aS)-1-[(S)-2-[[(S)-1-(ethoxycarbonyl)-3-phenylpropyl] amino] propanoyl] octahydro cyclopenta [b] pyrrole-2-carboxylic acid (Figure 1) is also used to treat hypertension, prevent strokes, heart attacks and kidney problems [4, 5, 8 and 9]. It is an angiotensin converting enzyme (ACE) inhibitors. ACE is an enzyme which produces the chemical angiotensin II. Angiotensin II is responsible for narrowing the arteries and elevating blood pressure. The most common side effects of Ramipril are: - abdominal pain, constipation, diarrhea, rash, dizziness, fatigue, headache, vomiting and loss of taste [2, 3, 4 and 5].

Amlodipine Besylate and Ramipril both are official in Indian Pharmacopeia and British Pharmacopeia. Literature survey revealed that various methods such as UV, HPLC, HPTLC, etc. are available in single and combination with other drugs [5, 6, 7 and 8]. However, no or only few spectrophotometric method has been reported for simultaneous estimation of Amlodipine Besylate and Ramipril in Tablet dosage forms. Hence an attempt has been made to develop and validate in accordance with ICH guidelines [9, 12,18,19,20 and 21].

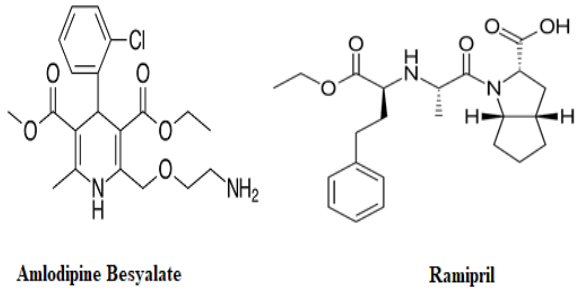

Figure 1: Chemical Structure of Amlodipine Besylate and Ramipril.

MATERIALS AND METHODS

Instrumentation and Reagents

SHIMADZU double beam UV visible spectrophotometer model 1800 and SHIMADZU analytical balance were used for measuring absorbance and weighing balance respectively. All the chemicals which we had used in this were of AR grade and manufactured by Central Drug House (P) Ltd. Distilled water and Whatman filter paper for filtration. Active pharmaceutical ingredients (API) Amlodipine Besylate (AB) and Ramipril were obtained as gift samples from M.M. College of Pharmacy, Mullana, Ambala and test samples were from tablet Ramistar $A_{2.5}$ (composition Aamlodipine Besylate 5mg and Ramipril 2.5mg) from Lupin Ltd., Jammu.

Preparation of Phosphate Buffer Solution 6.8 pH

6.8 gm of Potassium Dihydrogen Phosphate (KH_2PO_4) was taken and dissolved it in 1000 ml of distilled water and stirred and 0.9 gm of Sodium Hydroxide was mixed it well. It was showed exactly pH 6.8 no need to adjust pH.

Preparation of Standard Stock Solution

Amlodipine Besylate:

10 mg of Amlodipine Besylate was dissolved in 1 ml Methanol and 9 ml Buffer (Standard Stock Solution). 1 ml from the above solution was taken and poured it into 99 ml of Buffer to make a 10 μg/ml Solution (for Scanning).

Ramipril:

10 mg of Ramipril in 1 ml of Methanol and 9 ml Buffer (Standard Stock Solution). 5 ml of the above solution was taken and poured it into 99 ml of Buffer to make a 50 μg/ml solution (for Scanning).

Determination of Absorbance Maxima:

By using 10 μg/ml of Amlodipine besylate and 20 μg/ml of Ramipril solution find the λmax (nm) in UV Spectrophotometer.

Preparation of Calibration Curve

Diluted the Standard Stock Solution of Amlodipine Besylate and Ramipril with 6.8 pH buffers as solvent to get a series of concentration between 5-30 μg/ml of Amlodipine Besylate and 50-250 μg/ml of Ramipril. The Absorbance of each solution was measured at 238.1 nm and 250.9 nm in 1 cm cell against 6.8 pH Buffer as Blank. The graphs plotted as Concentration Vs Absorbance at selected wavelength for Amlodipine Besylate and Ramipril.

Preparation of Drugs Sample

Accurately weighed twenty tablets containing Ramipril 2.5 mg and Amlodipine besylate 5 mg with excipients. Then transferred into in a clean and dry mortar and pestle and crushed them into a fine powder. From the powder of twenty tablets accurately weighed the powder equivalent to single tablet, then transferred to the 50 ml Volumetric Flask to this 20 ml Methanol was added and for dissolving the drug used sonicator approximately for 10 minutes. Then passed it through the whatman filter paper and made up the volume up to 50 ml from 6.8 pH buffer. From this solution made a 10 μg/ml and 50 μg/ml solution for Amlodipine besylate and Ramipril respectively.

Simultaneous Estimation of Amlodipine Besylate and Ramipril.

In simultaneous method we used Absorbances at two selected wavelengths. To determine the λmax of both the drugs we scan in the range of 200-400 nm. Standard Solutions of different concentrations of both the drugs were prepared in phosphate buffer. Absorbance of Amlodipine Besylate (10 μg/ml) and Ramipril (50 μg/ml) were recorded at two wavelengths 238.1 nm and 250.9 nm by using Simultaneous Equation Method.

Cx = A2ay1-A1ay2/ax2ay1-ax1ay2 --------- (1)
Cy = A1ax2-A2ax1/ax2ay1-ax1ay1 --------- (2)

Where, A1 and A2= absorbance of the sample solution was measured at 238.1 nm and 250.9 nm respectively. x and y are Amlodipine besylate and Ramipril respectively. Cx and Cy = Concentration of Amlodipine Besylate and Ramipril respectively. ax1 and ax2 = absorptivity of Amlodipine Besylate at 238.1 nm and 250.9 nm. ay1 and ay2 = absorptivity of Ramipril at 238.1 nm and 250.9 nm.

METHODS VALIDATION

1. **Linearity:** It is the ability (in a certain range) to get test results in such a way that it is directly proportional to the concentration (amount) of analyte in the sample. The value of R should be close to 1.
2. **Precision:** It is the degree of agreement among individual test results when we applied the repeatedly to multiple sampling of homogenous samples.
3. **Accuracy:** it is the closeness of test results which we were obtained by our method to the true value.
4. **Limit of Detection (LOD) and Limit of Quantitation (LOQ) Determinations:** Limit of Detection (LOD) is the lowest concentration of analyte that can be detected, but not necessarily determined in a quantitative fashion by using specific method under the appropriate experimental conditions and Limit of Quantitation (LOQ) is a parameter of quantitative assays for low levels of compounds in samples matrices such as impurities in bulk drugs and degradation products in finished Pharmaceuticals.

RESULTS AND DISCUSSIONS

We have developed a simple, accurate and precise UV spectrophotometric method and also validated of marketed formulation which contained Amlodipine Besylate 5mg and Ramipril 2.5 mg by using Simultaneous Estimation Method. The λmax (nm) of Amlodipine Besylate was 238.1 nm and Ramipril was 250.9 nm and k*absorbance of Amlodipine Besylate at 238.1 nm was 0.309 and k*absorbance of Ramipril at 250.9 nm was 0.136. On putting these values in Simultaneous Equation Method Cx = A2ay1-A1ay2/ax2ay1-ax1 and Cy = A1ax2-A2ax1/ax2ay1-ax1ay1, we were found that the concentration of Amlodipine Besylate was 98.8% and the concentration of Ramipril was 98.48 % in sample or marketed formulation. The linearity range of Amlodipine Besylate and Ramipril were found to be 5-40 μg/ml and 50-250 μg/ml respectively. and the Regression Coefficient (R^2) for the same drugs was 0.998 and 0.999 respectively. The LOD and LOQ value of Amlodipine Besylate was found 0.157 and 0.483 respectively, whereas the LOD and LOQ value of Ramipril was found 0.056 and 0.145 respectively.

Table No 1. Determination of λmax for Amlodipine Besylate and Ramipril.

S.No	Name of the Drugs	Concentration (μg/ml)	λmax (nm)	K*Abs
1	Amlodipine Besylate	10	238.1	0.309
2	Ramipril	20	250.9	0.136

Table No 2. Dilution Study of Amlodipine Besylate for Calibration Curve.

S.No	Concentration μg/ml	Absorbance
1	5	0.154
2	10	0.309
3	20	0.462
4	30	0.616
5	40	0.770

Table No 3. Dilution Study of Ramipril for Calibration Curve.

S.No	Concentration μg/ml	Absorbance
1	50	0.136
2	100	0.203
3	150	0.270
4	200	0.330
5	250	0.408

Simultaneous Estimation of Amlodipine Besylate and Ramipril

Cx=A2ay1-A1ay2/ax2ay1-ax1ay2 --------- (1)
.Cy=A1ax2-A2ax1/ax2ay1-ax1ay1 --------- (2)

Where, A1 and A2 = Absorbance of the sample solution measured at 238.1 nm and 250.9 nm respectively. x and y are Amlodipine Besylate and Ramipril respectively. Cx and Cy = concentration of Amlodipine Besylate and Ramipril respectively. ax1 and ax2= absorptivity of Amlodipine Besylate at 238.1 nm and 250.9 nm. ay1 and ay2= absorptivity of Ramipril at 238.1 nm and 250.9 nm.
By using the values in equation.
Cx=A2ay1-A1ay2/ax2ay1-ax1ay2
= 9.88 μg/ml
For percentage = (9.88/10) (100)
= 98.8%

Cy = A1ax2-A2ax1/ax2ay1-ax1ay1
= 49.24 μg/ml
For percentage = (49.24/50) (100)
= 98.48%

Table No 4. Determination of Absorptivity for Amlodipine Besylate and Ramipril.

S.No	Name of the Drugs	Absorptivity				
		a	b	c	abc	ax
1	Amlodipine besylate	0.309	1	10	3.09	ax1
		0.18	1	10	1.8	ax2
2	Ramipril	0.026	1	50	1.3	ay1
		0.136	1	50	0.75	ay2

Where, a = absorbance, b = 1 (cell length in cm) and
c = concentration in μg/ml

Table No 5. Absorptivity Study for Amlodipine Besylate and Ramipril.

S.No	Name of the Drugs	A1 (238.1 nm)	A2 (250.9 nm)
1	Amlodipine Besylate	0.641	0.537
2	Ramipril	0.83	0.136

VALIDATION PARAMETERS

1. Linearity:

Consider any five points from both the standard calibration curve from 5-40 μg/ml and 50-250 μg/ml for Amlodipine besylate and Ramipril respectively. The drug responses were found to be linear and the linear regression of Amlodipine besylate was y = 0.031x - 0.001 with correlation coefficient 0.998 and the linear regression of Ramipril was y = 0.0013x + 0.0681 with correlation coefficient 0.9986.

Table No 6. Evaluation of Linearity for Amlodipine Besylate and Ramipril.

S.No	Name of the Drugs	Concentration (μg/ml)	Correlation Coefficient (R^2)
1	Amlodipine Besylate	5	0.998
		10	
		20	
		30	
		40	
2	Ramipril	50	0.999
		100	
		150	
		200	
		250	

2. **Precision:**

It is usually expressed as the standard deviation or the relative standard deviation (Coefficient of Variation).

Table No 7. Evaluation of Precision for Amlodipine Besylate and Ramipril.

S.No	Drug	Sample No.	% Assay	
		Set	Intraday	Interday
1	Amlodipine Besylate	1	98.8	99.4
		2	99.4	98.8
		3	99.1	99.9
		Mean	**99.1**	**99.36**
		SD	**0.244**	**0.397**
		%RSD	**0.226**	**0.399**
2	Ramipril	1	98.48	99.36
		2	99.36	98.94
		3	99.96	99.89
		Mean	**99.26**	**99.39**
		SD	**0.728**	**0.4761**
		%RSD	**0.733**	**0.479**

3. Accuracy:

It was done by standard addition method at 3different levels.

Table No 8. Evaluation of Accuracy for Amlodipine Besylate and Ramipril.

S.No	Name of the Drugs	Amount of Standard added (μg/ml)	Mean % Recovery	SD
1	Amlodipine Besylate	1.5	98.8	0.551
		2	99.32	0.619
		2.5	99.89	0.695
2	Ramipril	10	98.4	0.451
		20	99.03	0.526
		30	99.58	0.579

*****Average of Three Estimations

4. Limit of Detection (LOD) and Limit of Quantitation (LOQ) Determinations:

The Limit of Detection and Limit of Quantitation were calculated as 3 times and 10 times the noise level respectively.

Table No 9. Evaluation of Limit of Detection (LOD) and Limit of Quantitation (LOQ) of Amlodipine Besylate and Ramipril.

S.No	Name of the Drugs	LOD	LOQ
1	Amlodipine Besylate	*0.157*	0.483
2	Ramipril	0.056	0.145

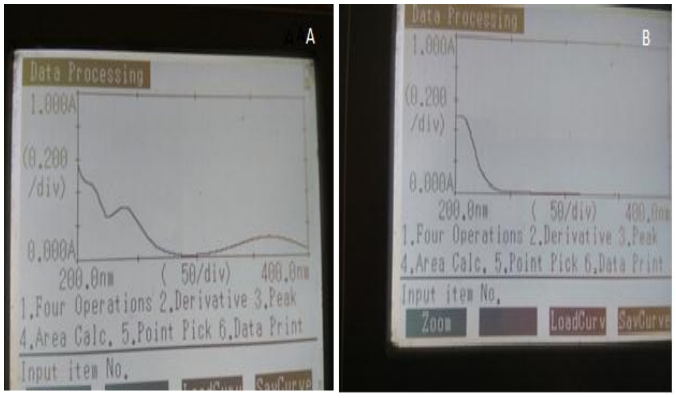

Figure 2: Scanning of (A) Amlodipine Besylate and (B) Ramipril.

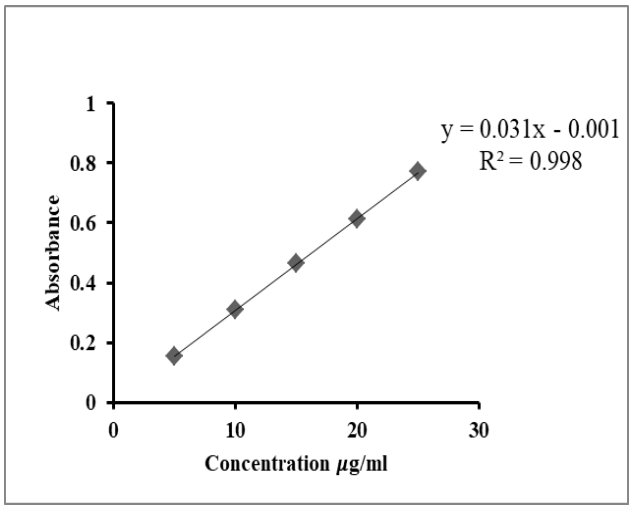

Figure 3: Calibration Curve of Amlodipine Besylate.

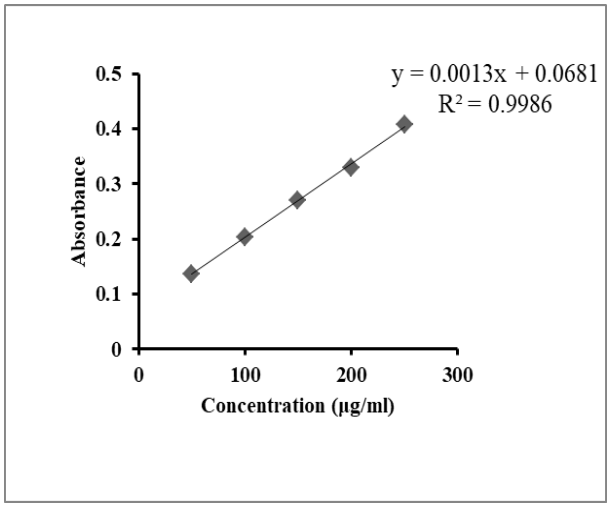

Figure 4. Calibration Curve of Ramipril

CONCLUSIONS

The method which we have performed is specific, accurate and precise for the simultaneous estimation of Amlodipine Besylate and Ramipril. The method which we have developed as per ICH guidelines and the method can be applied for daily routine analysis in Quality Control Laboratories.

REFERENCES

1. http://en.wikipedia.org/wiki/Amlodipine.
2. http://en.wikipedia.org/wiki/Ramipril.
3. Tripathi, K. D. *Essential of Medical Pharmacology* Jaypee Brothers Medical Publishers, New Delhi. 2003
4. Joel G. H., Lee E. L. *Goodman and Gilman's. The Pharmacological Basis of Therapeutics*, The MC Graw Hill companies Inc, New York. 2001
5. Walker R., Edwards C. *Clinical Pharmacy and Therapeutics*. Churchill Livingstone Longman Singapore publishers Ltd. Singapore 1994.
6. Rahman N., Azmi S.N.H. Kinetic Spectrophotometric Method for the Determination of RAM in Pharmaceutical Formulations, *AAPS Pharm. Sci. Tech.*, 2005, 6(3), E543-E551.
7. Aboul Enein H. Y., Raluca I. S, Jacobus F V. Analysis of several angiotensin-converting enzyme inhibitors using potentiometric, enantioselective membrane electrodes. *Anal. 1. Lett.* 1999; 32: 623–632.
8. Bonazzi D, Gotti V, Andrisano V, Cavrini V. Analysis of ACE Inhibitors in Pharmaceutical Dosage Forms by Derivative UV Spectroscopy and Liquid Chromatography (HPLC). *J. Pharm. Biomed. Anal*. 1997; 16: 431-438.
9. Sridhar K, Sastry CSP, Reddy MN. Spectrophotometric Determination of Amlodipine Besylate in pure forms and tablets. *Analytical letters*. 1997; 30: 121- 126.
10. Mishra P, Gupta Alka, Shah K., Simultaneous Estimation of Atorvastatin Calcium and Amlodipine Besylate from Tablets, 2007, 69, 831-833.
11. Singhvi I. Chaturvedi S.C., Spectrophotometric Method for Estimation of Amlodipine Besylate and Benidipine Hydrochloride from Tablets, *Indian Journal of Pharmaceutical Sciences*, 1999, 190-192.
12. Wankhede S. B., Wadkar S. B., Raka K. C., Chitlage S. S., Simultaneous estimation of Amlodipine Besylate and Olmesartan in Pharmaceutical Dosage Form, *Indian Journal of Pharmaceutical Sciences*, 2009, 563-567.
13. Saravanan C, Srinivasan S, Narayanaswamy VB, Suresh S, Sivakuma T. Analytical Method Development and Validation of Amlodipine Besylate and Losartan Potassium in Tablet Dosage Form by RP-HPLC method. *Int J Pharm Sci* 2010;2(3):822-6.
14. Patil PR, Rakesh SU, Dhabale PN, Burade KB. Simultaneous UV Spectrophotometric Method for Estimation of Losartan Potassium and Amlodipine Besylate in Tablet Dosage Form. *Asian J Res Chem* 2009; 2(1):183-7.
15. Patil PR, Rakesh SU, Dhabale PN, Burade KB. RP-HPLC Method for Simultaneous Estimation of Losartan Potassium and Amlodipine Besylate in Tablet Formulation. *Int J Chem Tech Res* 2009;1(3):464-9.
16. Garg G, Saraf S, Saraf S. Development and Validation of Simultaneous Estimation of Enalapril Maleate and Amlodione Besylate in Combined Dosage Forms. *Trends Appl Sci Res* 2008;3(3):278-84.
17. S. Bankey, G. G Tapadiya, S. S Saboo, S. Bindaiya, Deepti Jain, S. S. Khadbadi: Simultaneous Determination of Ramipril, Hydrochlorothiazide and Telmisartan by Spectrophotometry, *Inter J of Chem Tech Research* 2009; 1:183-188.
18. Kakde RB, Kotak VH, Barsagade AG, Chaudhary NK,. Spectrophotometric Method for Simultaneous Estimation of Amlodipine Besylate and Bisoprolol Fumarate in Pharmaceutical Preparations. *Research J Pharm Tech* 2008 1:513-515.
19. Kardile DP, Kalyane NV, Thakkar TH, Patel MR, et al. Simultaneous Spectroscopic Estimation of Amlodipine Besylate and Olmesartan Medoxomil Drug Formulations by HPLC and UV-Spectrophotometric Method. *J Pharm Sci Res* 2010 2:599-614.
20. ICH Harmonised Tripartite Guidelines. Validation of Analytical Procedures: Text & Methodology, Q2 (R); Nov, 2005.
21. ICH, Validation of Analytical Procedures: Methodology Q2 (R1), International Conference on Harmonization, IFPMA, Geneva, 1996.