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

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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=0.4291x0.0084 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.

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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 benzenesulfonic 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 nonsulfhydryl 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;

Karajgi and Kulkarni, 2013). This put to an end the potent vasoconstrictive actions of angiotensin II and leads to vasodilatation (Jampana *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.

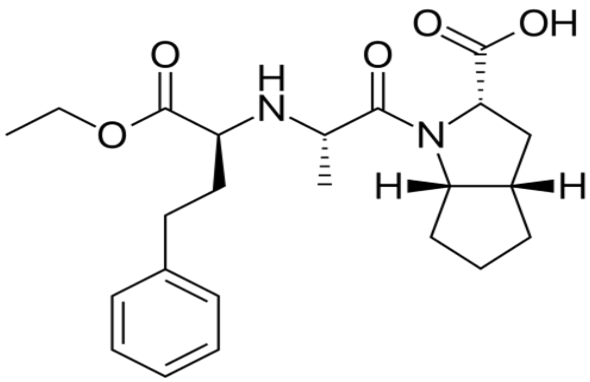

Figure 1: Chemical structures of Ramipril

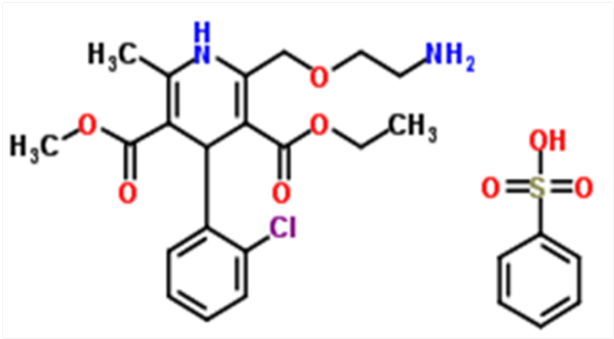

Figure 2: Chemical structures of Amlodipine besylate

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.

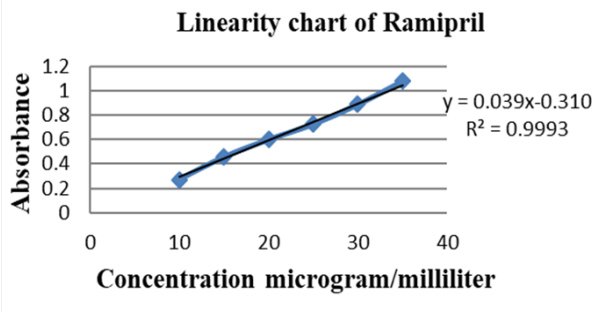

Figure 3: Linearity chart of Ramipril

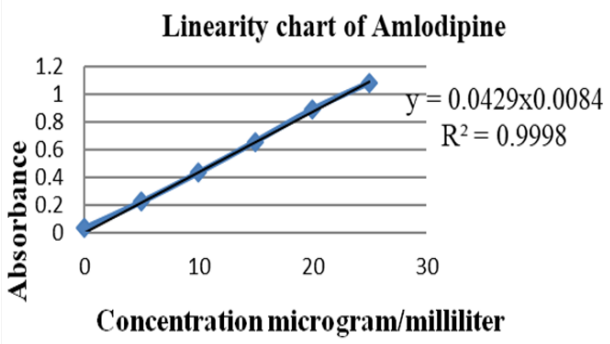

Figure 4: Linearity chart of Amlodipine

Standard stock solution Preparation

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

Selection of Wavelength

For estimation of Ramipril employing 210 nanometer as wavelength was fixed. Forthe 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 filter paper, discarding first few ML. It is diluted till the mark by methyl alcohol to obtain stock strength of 50 μg/mL of Ramipril and 100 μg/mL of Amlodipine besylate.

RESULTS AND DISCUSSION

Method development

Amlodipine besylate and Ramipril standard com-

Table 1: Linearity table of Amlodipine and Ramipril

S.No.	Ramipril		Amlodipine besylate	
	Conc(μg/mL)	Absorbance	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

	Intra-day precision			Inter-day precision		
S. No.	Time (Hours)	Ramipril Absorbance	Amlodipine Absorbance	Time (Days)	Ramipril Absorbance	Amlodipine Absorbance
1	0	99.58	99.85	1	99.75	99.74
2	2	99.68	99.98	2	98.98	99.69
3	4	99.78	99.85	3	99.69	99.88
4	6	99.89	99.64	4	99.87	99.87
5	8	99.86	99.87	5	98.79	99.92
6	10	99.89	99.68	6	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

	Ramipril			Amlodipine besylate		
	80%	100%	120%	80%	100%	120%
Std. conc. (μg/mL)	10	10	10	10	10	10
Conc. added (μg/mL)	8	10	12	8	10	12
Conc. found (μg/mL)	7.95	9.91	11.94	7.97	9.98	11.92
% Recovery	99.375	99.1	99.5	99.62	99.8	99.33
% Mean recovery	99.32				99.58	

Table 4: Stability studies parameters for Ramipril and Amlodipine besylate

Sample (treated)	% Assay	
	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 measured value(μg/mL)	Amlodipine besylatemeasured value(μg/mL)
Limit of detection	0.25	0.37
Limit of quantification	0.34	0.97

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

pound were taken for dilution 100μg/ml. Different dilution was taken from standard stock solution 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

Theextent 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

None.

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