METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF RAMIPRIL AND FELODIPINE BY UV SPECTROPHOTOMETRIC METHOD

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ABSTRACT:

A simple, accurate, rapid and precise UV Spectrophotometric method has been developed using methanol as a solvent for the simultaneous estimation of Ramipril (RAM) and Felodipine (FEL) in pharmaceutical dosage form. Ramipril exhibits absorption maximum at 210 nm and Felodipine shows absorption maximum at 237 nm. The Linearity was observed in the concentration range of 10-60 µg/ml for RAM and FEL. The precision of method was determined by performing intra-day and inter-day study. The accuracy of method was confirmed by recovery studies. The analytical method was validated for various parameters as per ICH guidelines. The proposed method was found to be simple and can be used for the routine analysis for estimation of RAM and FEL in bulk and tablet dosage forms.

Key words: Ramipril, Felodipine, UV Spectrophotometric method, Dosage form

INTRODUCTION:

Ramipril (Fig. 1) is chemically (2S,3aS,6aS)-1-[(S)-2-[[(S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino] propanoyl] octahydrocyclopenta [b]pyrrole-2-carboxylic acid[1,2]. It is an ACE inhibitor, indicated for the treatment of hypertension, heart failure and myocardial infarction. Angiotensin converting enzyme is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex. Ramipril lowers blood pressure by inhibiting angiotensin-converting enzyme (ACE) and is believed to be primarily suppression of the renin-angiotensin-aldosterone system.

Felodipine (Fig. 2) is chemically Ethylmethyl-4-(2, 3-dichlorophenyl) 1,4-dihydro-2,6-dimethyl-3,5–pyridine dicarboxylate[3,4]. Felodipine is a dihydropyridine calcium channel blocker, indicated for the treatment of hypertension and angina pectoris. In- vitro studies show that the effects of Felodipine on contractile

processes are selective, with greater effects on vascular smooth muscle than cardiac muscle[5]. Felodipine reversibly competes for dihydropyridine binding sites, blocks voltage-dependent Ca^{++} currents. The approach of combination therapy may be theoretically favored by the fact that multiple factors contribute to the hypertension and achieving control of BP with single agent that acts through one particular mechanism may be unrealistic. Combining the second agent may lead to better control, acting by complimentary mechanism.

Literature survey reveals very few analytical methods like Spectroflourimetric[6] UV Spectroscopy[7,8] and HPLC[9]methods are reported for the simultaneous determination of Ramipril and Felodipine in combined dosage form. Present work emphasizes on the quantitative estimation of Ramipril and Felodipine in their combined dosage form by UV Spectroscopic method.

MATERIALS AND METHODS

Chemicals & Reagents:

The reference samples of Ramipril (API) and Felodipine (API) were provided as gift samples from Spectrum Labs, Hyderabad, India. The commercial formulations (tablets) (TRIASYN tablets containing 5 mg of Ramipril and 5 mg of Felodipine) were procured from the local market.

Methanol (AR grade) was purchased from E.Merck (India) Ltd., Mumbai, India and was used as solvent. Fresh purified distilled water was used throughout the experiment.

Instrument:

Shimadzu UV1800 Double Beam UV-Visible Spectrophotometer was used for spectral studies. Shimadzu BL220H Digital Weighing Balance was used for weighing the materials.

Determination of wavelength of maximum absorption:

Pure samples of both Ramipril and Felodipine (100 mg) were dissolved in 100 ml of methanol to give standard solutions of 1000 µg/ml concentration. The solutions were further diluted to give the resultant concentration of 10 µg/ml. These solutions were scanned in the wavelength region of 200-400 nm and the λmax was found to be 210 nm for Ramipril and 237 nm for Felodipine respectively.

Linearity Study:

Working standard solutions were prepared for the Ramipril and Felodipine from the standard solution of 100 µg/ml. Different aliquots were taken from standard stock solution and diluted with methanol separately to prepare 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml and 60 µg/ml solutions respectively. Prepared working solutions of Ramipril and Felodipine were scanned at 210 nm and 230 nm respectively. The calibration curves were plotted by taking their respective absorbances against the concentrations.

Assay of tablet dosage form:

Twenty tablets were procured from local market and average weight was determined. The powder sample equivalent to 5 mg of Ramipril and 5 mg of Felodipine was weighed accurately and transferred into a 100 ml clean dry volumetric flask containing 70 ml of methanol. The solution was sonicated for 5 min and the drug was dissolved completely. Further pipette 2 ml of the above stock solution into a 10 ml volumetric flask and the volume was made up to the mark with the methanol.

RESULTS:

The present work provides an accurate, sensitive and precise spectrophotometric method for the simultaneous analysis of Ramipril and Felodipine in bulk samples and their tablet dosage forms. The selected wavelengths in methanol for estimation of Ramipril was found to be 210 nm and for Felodipine was found to be 237 nm. Linear relationships between drug concentrations were obtained over the range of 10-60 µg/ml for both Ramipril (Fig. 3) and Felodipine (Fig. 4). The regression equations of Ramipril and Felodipine over its absorbances were found to be y=0.0096x+0.0315 and y=0.0102x+0.0218 (Table 1) respectively with a correlation coefficient (r^2) of 0.9911 for Ramipril and 0.9948 for Felodipine. The %RSD for intra-day precision and inter-day precision for Ramipril were found to be 0.29% and 0.10% respectively .The %RSD for intra-day

precision and inter-day precision for Felodipine were found to be 1.96% and 0.05% respectively (Table 2& 3).This reveals that the method is quite precise. The percent recoveries of the drug solutions of Ramipril and Felodipine were studied at three different concentration levels. Satisfactory results were obtained with the mean recovery of 98.45% and 98.52% for Ramipril (Table 4) and Felodipine (Table 5) respectively. The limit of detection (LOD) and limit of quantification (LOQ) for Ramipril were found to be 0.30 µg/ml and 0.92 µg/ml respectively (Table 6). The limit of detection (LOD) and limit of quantification (LOQ) for Felodipine were found to be 0.57 µg/ml and 1.75 µg/ml respectively (Table 6). The percentage purity for the assay of Ramipril and Felodipine were found to be 99.68% and 100.04 % (Table 7) respectively. The method shows no interference from common excipients and additives in tablet formulations with the simultaneous estimation of the drugs Ramipril and Felodipine by the proposed UV spectrophotometric method.

CONCLUSION:

The proposed method for the simultaneous determination of Ramipril and Felodipine in bulk and tablet dosage forms was found to be simple, sensitive, precise and accurate. Both drugs showed good regression values at their respective wavelengths and the results of recovery study revealed that any small change in the drug concentration in

the solution could be accurately determined by the proposed method and low values of LOD and LOQ indicated good sensitivity of proposed methods. Hence proposed methods are new, simple, accurate, sensitive and precise and can be adopted for rapid determination of Ramipril and Felodipine in tablet dosage form.

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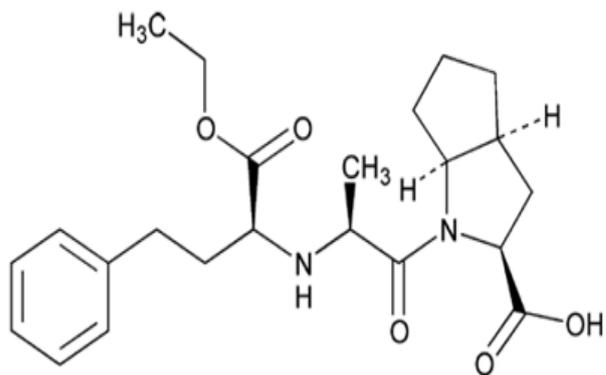
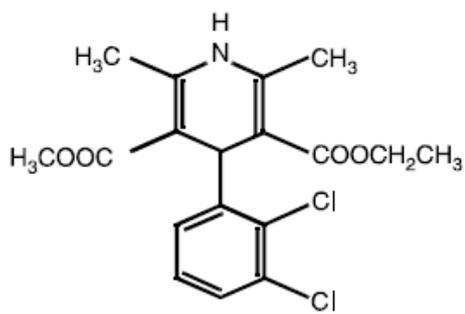**Fig. 1: Molecular structure of Ramipril**
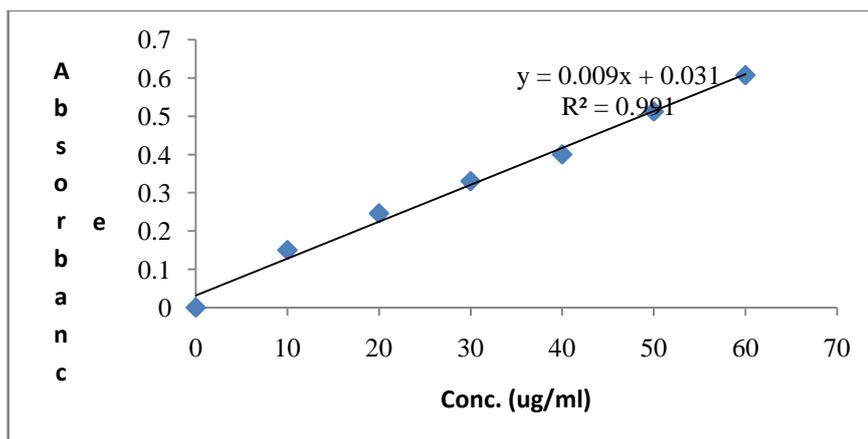

Fig. 2: Molecular structure of Felodipine

$y = 0.009x + 0.031$
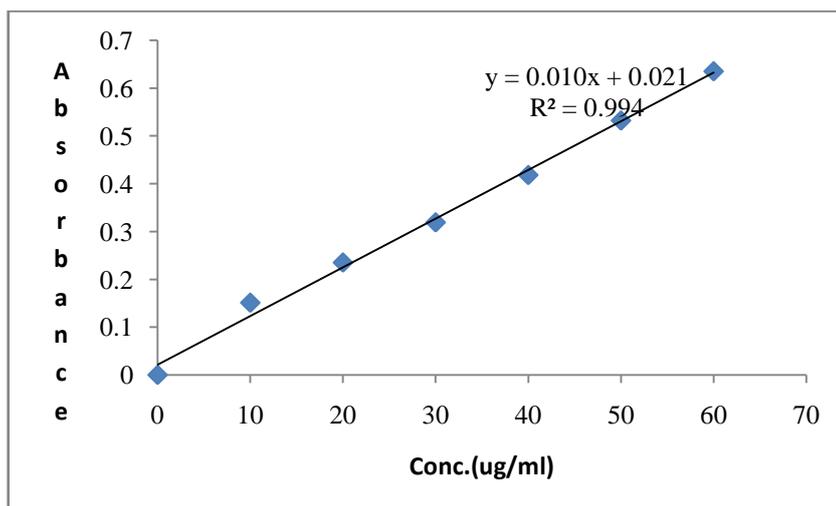$R^2 = 0.991$

Fig. 3: Calibration curve of Ramipril

Fig. 4: Calibration curve of Felodipine

Table 1: Linearity results of Ramipril and Felodipine

S. No.	Concentration (µg/ml)	RAM Absorbance	FEL Absorbance
1	0	0	0
2	10	0.15	0.151
3	20	0.246	0.235
4	30	0.33	0.319
5	40	0.4	0.418
6	50	0.512	0.532
	60	0.607	0.635
Slope		0.0096	0.0102
Intercept		0.0315	0.0218
Regression Equation (y)		0.0096x+0.0315	0.0102x+0.0218
Correlation Coefficient (r^2)		0.9911	0.9948

Table 2: Intra-day precision results of Ramipril and Felodipine

S. No.	Time (Hours)	RAM Absorbance	FEL Absorbance
1	0	0.302	0.304
2	3	0.301	0.304
3	6	0.301	0.302
4	9	0.303	0.3
5	12	0.302	0.302
6	15	0.303	0.3
	Mean	0.302	0.302
	SD	0.00089	0.001788

	%RSD	0.29	1.96

Table 3: Inter-day precision results of Ramipril and Felodipine

S. No.	Time (Hours)	RAM Absorbance	FEL Absorbance
1	0	0.302	0.301
2	3	0.301	0.301
3	6	0.302	0.3
4	9	0.302	0.302
5	12	0.3	0.304
6	15	0.302	0.302
	Mean	0.3015	0.3016
	SD	0.000316	0.000017
	%RSD	0.1	0.05

Table 4: Recovery studies for Ramipril

Level	Standard conc. (µg/ml)	Conc. added (µg/ml)	Conc. found (µg/ml)	% Recovery	% Mean recovery
50%	10	5	4.95	99.00	
100%	10	10	9.77	97.70	98.45
150%	10	15	14.80	98.66	

Table 5: Recovery studies for Felodipine

Level	Standard conc. (µg/ml)	Conc. added (µg/ml)	Conc. found (µg/ml)	% Recovery	% Mean recovery
50%	10	5	4.96	99.20	
100%	10	10	9.77	97.70	98.52
150%	10	15	14.8	98.66	

Table 6: LOD and LOQ of Ramipril and F elodipine

Parameter	Measured value (µg/ml)	
	Ramipril	Felodipine
Limit of detection	0.30	0.57
Limit of quantification	0.92	1.75

Table 7: Assay results of Ramipril and Felodipine formulations

Formulation		Label claim	Amount found	%Assay
TRIASYN	Ramipril	5 mg	4.984 mg	99.68%
	Felodipine	5 mg	5.002 mg	100.04%