Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(2):1383-1388

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Simultaneous high-performance liquid chromatographic determination of enalapril and felodipine in pharmaceutical-dosage form

Sai Chaitanya[1*] B, Satish babu K[1], Satish kumar V[1], Ravindra babu SVD[2]

[1]*Vishwabharathi College of Pharmaceutical Sciences, Perecherla, Guntur, India*
[2]*SRM College of Pharmacy, Chennai, India*

ABSTRACT

A simple, rapid and precise method is developed for the quantitative simultaneous determination of Enalapril and Felodipine in a combined pharmaceutical-dosage form. The method is based on High Performance Liquid Chromatography (HPLC) on a reversed-phase column, shim-pack CLC, ODS (C18), 4.6 mm × 25 cm & 0.5 µm, using a mobile phase of ammonium acetate buffer (the pH was adjusted to 4.5 ± 0.05 with glacial acetic acid), acetonitrile and methanol (35:30:35 v/v). The buffer used in the mobile phase contains ammonium acetate in double-distilled water. The chromatographic conditions are- flow rate of 1.5ml/min, column temperature at 40°C and detector wavelength of 237 nm. Both the drugs were well resolved on the stationary phase and the retention times were around 1.5 minute for Enalapril and 3.4 minute for Felodipine. The method was validated and shown to be linear for Enalapril and Felodipine. The correlation coefficients for Enalapril and Felodipine are 0.999963 and 0.999979, respectively. The relative standard deviations for six replicate measurements in two sets of each drug in the tablets is always less than 2% and mean % error of active recovery not more than ± 1.5%. The method was validated for precision and accuracy. The proposed method was successfully applied to the pharmaceutical dosage forms containing the above-mentioned drug combination without any interference by the excipients.

Keywords: Enalapril and Felodipine, Validation, HPLC, tablets.

INTRODUCTION

Fixed-dose combination of antihypertensive drugs can simplify dosing regimens, improve compliance, improve hypertension control, decrease dose-dependent side effects and reduce cost as the first-line treatment of hypertension [1]. These potential advantages make it recommendable for the combination antihypertensive therapy to be used as initial treatment, particularly in patients with target-organ damage or more severe initial hypertension[2, 3]. Calcium antagonists are vasodilatory and tend to increase plasma renin, therefore combination with a β-blocker is theoretically sound [4]. Felodipine, with its intrinsically long half-life alone or together with β-blocker, is likely to produce superior ischaemia reduction in clinical practice when patients frequently forget to take medication or take doses irregularly [5, 6, 7] found that adding Felodipine to Enalapril produced a significant reduction in blood pressure when compared with placebo in patients whose blood pressure was not controlled by Enalapril alone. The reduction of side-effects, obtained by adding a dihydropyridine derivate to a β-blocker, confirms the effectiveness of this combination [7]. It is clearly demonstrated that the combination of Enalapril and Felodipine is synergistic in lowering and stabilizing BP and this synergism is highest when the dose proportion of the two drugs is 10 : 1 [8]. The Indian Pharmacopoeia describes non-aqueous titration method for the assay of Enalapril. The British Pharmacopoeia examines Felodipine besylate by liquid chromatography. An UV-spectrophotometric [9] and

reversed phase HPLC [10] methods are reported for simultaneous estimation of Enalapril and Felodipine besylate in combined dosage form, but to the best of our knowledge, no report related to the determination of Enalapril and Felodipine besylate in pharmaceutical dosage forms using a reversed-phase column, shim-pack CLC, ODS (C18), 4.6 mm × 25 cm & 0.5 μm, using a mobile phase of ammonium acetate buffer acetonitrile and methanol (35:30:35 v/v), has, so for, been mentioned in literature or in pharmacopoeias and hence the present work was undertaken.

The focus of the present study was to develop and validate a economic, rapid reversed-phase high performance liquid chromatographic method for the quality control of Enalapril and Felodipine besylate in pharmaceutical preparations with lower solvent consumption along with the short analytical run time leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. The proposed method is applicable as well as for routine analysis and content uniformity test of Enalapril and Felodipine besylate in tablets and complies well with the validation requirements in the pharmaceutical industry.

EXPERIMENTAL SECTION

Equipment and chromatographic conditions A high-performance liquid chromatographic system consisted of a pump LC-10 AS, oven CTO-10A, detector SPD-10A and recorder C-R6A (Shimadzu, Japan). All pH measurements were performed on a pH meter (Sentron, Netherlands). Chromatographic separation was carried out at room temperature with shim-pack CLC, ODS (C18), 4.6 mm × 25 cm & 0.5 μm column from Shimadzu, Japan. For the mobile phase, 1.54 gm of ammonium acetate was dissolved in 900 ml of double-distilled water. The buffer solution was shaked manually to dissolve and finally make the volume up to 1000 ml with the same. A mixture of ammonium acetate buffer acetonitrile and methanol in the ratio of 35: 30: 35 was prepared. The pH of the ammonium acetate buffer was adjusted to 4.5 ± 0.05 with glacial acetic acid. Finally the mobile phase was filtered through a 0.45 μm membrane filter and degassed for 10 minutes. The injection volumes for samples and standards were 20 μl and eluted at a flow rate of 1.5 ml/min at 40 °C. The eluents were monitored at 237 nm.

Materials and reagents

Acetonitrile and methanol were of HPLC grade and were purchased from E. Merck. Ammonium acetate, glacial acetic acid and other reagents were of analytical-reagent grade and purchased from E. Merck. Water was deionised and double distilled. Lexxel Plus tablets were kind gift from Square Pharmaceuticals Ltd. Each tablet was labeled to contain 50 mg Enalapril and 5 mg Felodipine. The excipients present in the tablets are: microcrystalline cellulose, maize starch, sodium starch glycolate, pigments blend-24843 (pink), colloidal silicon dioxide, magnesium stearate and purified talc.

Preparation of standard solutions

A working standard solution containing Enalapril 25 mg/100ml and Felodipine 2.5 mg/100ml was prepared by dissolving Enalapril and Felodipine besylate reference standard in mobile phase. The mixture was sonicated for 5 minutes or until the reference standard dissolved completely.

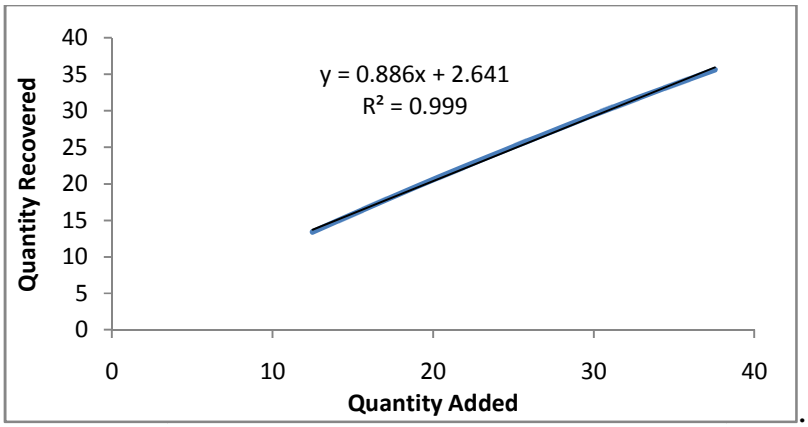
Preparation of sample solutions

Twenty tablets, each containing 50 mg Enalapril and 5 mg Felodipine were accurately weighed and finely powdered. A quantity of powder equivalent to 25 mg of Enalapril and 2.5 mg of Felodipine was weighted and transferred to a 100 ml volumetric flask. About 70 ml of mobile phase was added and shaked mechanically for 15 minutes. The mixture was then sonicated in ultrasonic bath for 5 minutes and makes the volume up to 100 ml by the mobile phase. The solution was filtered with a Whatman filter paper no.1. Before injection, both standard and sample solution was filtered through 0.45 μm syringe filter. Then 20 μl of standard and sample solutions were injected into column and chromatogram was recorded.

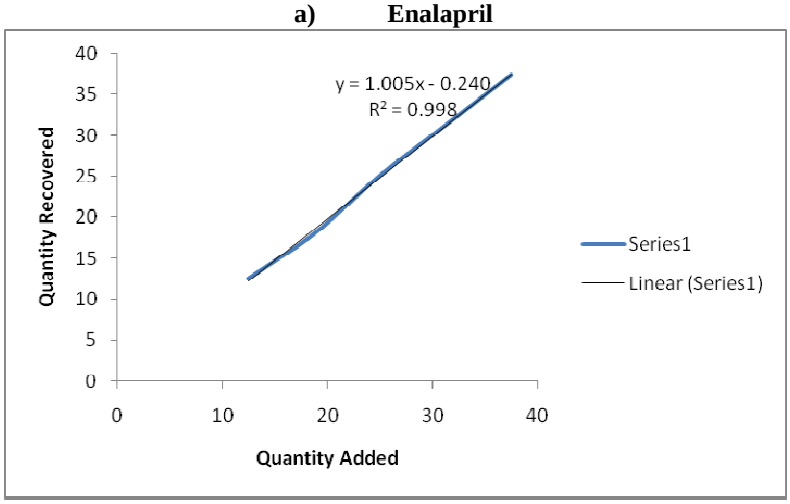
Method validation

The linearity of the analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity is important to demonstrate that the response of the measurement of detector system is linear over the range of interest of the method. This was determined by means of calibration graph using increasing amounts of a standard solution (0, 50, 75, 100, 125 and 150%) of both Enalapril and Felodipine. These standards were tested six times in agreement to the International Conference on Harmonization (ICH). A calibration curve was constructed and the proposed method was evaluated by its correlation coefficient and intercept value, calculated in the corresponding

statistical study (ANOVA) ($p < 0.05$). Characteristic parameters for regression equation ($y = a + bx$) of the HPLC method obtained by least squares treatment of the results was used to confirm the good linearity of the method developed. The correlation co-efficient between concentration and Peak Area found must not be less than 0.995. The accuracy of the assay was measured by analyzing five spiked samples of Enalapril and Felodipine (50, 75, 100, 125, 150%). The accuracy of this method was the closeness of the test results obtained by that method to the true value and established across its range. Accuracy was determined by means of recovery experiments, by spiked addition of active drug to placebo formulations. It was shown that the recoveries were independent of the concentration of the active over a reasonable concentration range normally 50 to 150 % of the nominal concentration. The amount recovered was plotted against the theoretical amount which produced a straight line of slope one and intercept zero. The accuracy was expressed by mean percentage error which was also to be determined and it was not more than ± 1.5 %. According to the ICH recommendations, precision must be considered at two levels, repeatability and intermediate precision. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment. On that account, a six-sample replicates were consecutively tested in the same equipment at a concentration of 100% of both Enalapril and Felodipine of the regular analytical working value. The intermediate precision expresses the with in laboratory variations, was assessed by using different equipments, analysts and days to analyze three samples six times. The relative standard deviation (% RSD) was determined in order to assess the precision of the assay and it was not be more than 2.0 %.



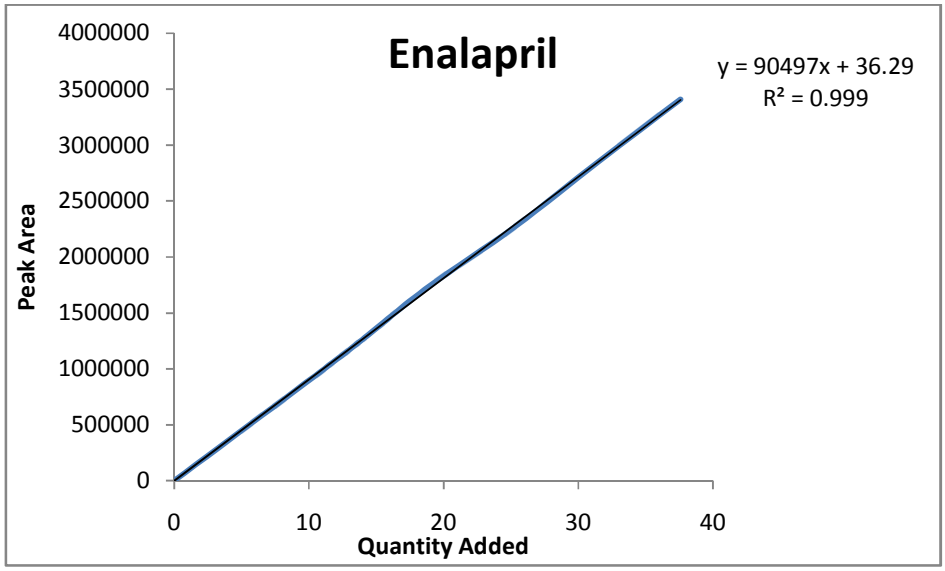
a) Enalapril



b) Felodipine

Fig. 1: Accuracy curves of Lexxel Plus tablet; (a) Enalapril (b) Felodipine.

[a)]The experiment was performed using C18 as the column of choice and ammonium acetate buffer solution (pH 4.5) mixed with acetonitrile and methanol (35:30:35) as mobile phase.



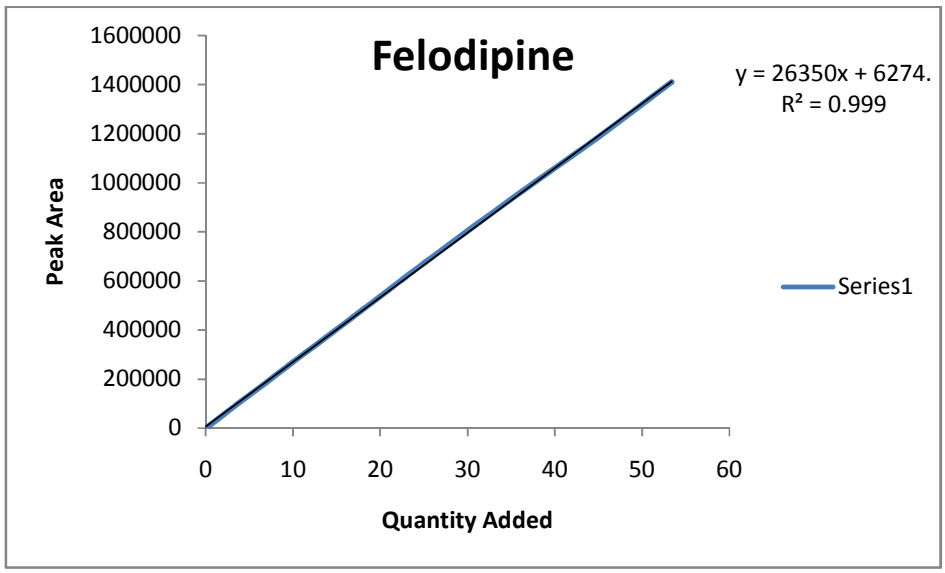


Fig. 2: Calibration curves of Lexxel Plus tablet; (a) Enalapril (b) Felodipine.

[b)]The experiment was performed using C18 as the column of choice and ammonium acetate buffer solution (pH 4.5) mixed with acetonitrile and methanol (35:30:35) as mobile phase.

Table 1: Summary of the results of Amount added vs Amount recovered

S.No	Enalapril		Felodipine	
	Active added (in mg)	Active recovered (in mg)	Active added (in mg)	Active recovered (in mg)
1	12.49	13.42±0.25	12.42	12.54±0.12
2	18.74	19.42±0.62	17.71	17.99±0.06
3	25.08	25.14±0.11	24.92	25.04±0.21
4	31.22	30.48±0.31	31.27	31.28±0.16
5	37.55	35.64±0.04	37.48	37.40±0.31

**Values are mean ± SEM of three determinations*

Table 2: Percent of active recovered from different % of sample

	Enalapril	Felodipine
Active Recovered from 50% Sample (in %)	105.52%	100.38%
Active Recovered from 75% Sample (in %)	103.88%	100.05%
Active Recovered from 100% Sample (in %)	100.52%	100.25%
Active Recovered from 125% Sample (in %)	97.22%	99.78%
Active Recovered from 150% Sample (in %)	92.92%	99.77%
Average mean of Recovery	100.82%	100.02%
Mean of error	0.828%	0.0275%

Table 3: Summary data of precision of the Enalapril and Felodipine HPLC determination

Precision								
Test		Mg/Tablet						%RSD
Repeatability		S1	S2	S3	S4	S5	S6	
	Enalapril	49.25	49.28	49.44	49.40	49.42	49.45	0.20185
	Felodipine	4.952	4.946	4.953	4.950	4.955	4.942	0.20833

[a] *The experiment was performed using C18 as the column of choice and ammonium acetate buffer solution (pH 4.5) mixed with acetonitrile and methanol (35:30:35) as mobile phase.*

Table 4: Summary of the results of amount added vs peak area

Sample No.	Different % of Sample	Enalapril		Felodipine	
		Active added (in mg)	Peak area	Active added (in mg)	Peak area
1	0%	0	0	0	0
2	50%	12.4	1113948	17.5	470198
3	75%	18.6	1707705	26.4	709344
4	100%	25.3	2269161	35.4	943691
5	125%	31.5	2850194	44.6	1175916
6	150%	37.6	3407556	53.4	1410375
Correlation coefficient		0.999966		0.999974	

Table 5: Determination of Enalapril and Felodipine in commercial formulations by high-performance liquid chromatography

Sample No.	Enalapril (mg/tablet)	Felodipine (mg/tablet)
Sample 1	49.54±0.92	4.95±0.25
Sample 2	50.26±1.10	5.04±0.44
Sample 3	47.96±1.14	5.02±0.41
Sample 4	49.65±0.47	4.97±0.23
Sample 5	49.42±0.75	4.85±0.66
Sample 6	48.90±0.85	4.99±0.38

Results are the mean ± S.E.M. of three independent experiments.

RESULTS AND DISCUSSION

Methods development and optimization

This isocratic-mode method with UV detection was developed for the determination of the active ingredients, Enalapril and Felodipine, at 100% level. Firstly, the reversed-phase column, shim-pack CLC, ODS (C18), 4.6 mm × 25 cm & 0.5 μm was tested. The system suitability studies were carried out as specified in ICH. The mobile phase consisted of ammonium acetate buffer solution acetonitrile and methanol at various ratios (40:30:30, 35:30:35, 30:40:30 (v/v)) was tested as starting solvent. The variation at the mobile phase leads to considerable changes in the chromatographic parameters. However, the proportion buffer: acetonitrile: methanol at a ratio of 35/30/35 (v/v) yielded the best results. Our data showed that the variation of the pH (3.5, 4.0, 4.5) of the ammonium acetate buffer did have significant effects on the HPLC-UV chromatographic resolution. Although the retention times of Enalapril and Felodipine showed a change in the pH variation (3.0–4.5), it was necessary to maintain pH value of the buffer at 4.5 for the optimum separation of the compounds, as at this pH the analyte peaks were well defined and resolved. In order to study the effect of excipients on quantification of Enalapril and Felodipine, a placebo was prepared using microcrystalline cellulose, maize starch, sodium starch glycolate, pigment blend-24843 (pink), colloidal silicon

dioxide, magnesium stearate and purified talc. The results revealed no interference of the excipients. In order to obtain a satisfactory and full detection for this new method, 3D-UV–vis spectra of standard Enalapril and Felodipine solution were obtained (data not shown). Based on the highest UV absorbance for Enalapril and Felodipine, 237 nm was chosen for detection of this new HPLC method at which the best detector responses for all substances were obtained.

Method validation

The accuracy was evaluated by the recovery of Enalapril and Felodipine at five different levels (50, 75, 100, 125 and 150%). An accuracy curve (fig. 1) was constructed and the summary of the results and average mean of recovery data for each level of both active pharmaceutical ingredients (API) was within accepted values was shown in tables 1 and 2. The data of table 3 showed that average results of repeatability of Enalapril and Felodipine and was within the limit and R.S.D. was 0.20188 and 0.20837, respectively, which indicated a good precision. A calibration curve (fig. 2) was constructed and the proposed method was evaluated by its correlation coefficient (0.999963 and 0.999979) (table 4). Characteristic parameters for regression equation (y = a + bx) of the HPLC method obtained by least squares treatment of the results confirmed the good linearity of the method developed (fig. 2). Label claim recoveries from Lexxel plus tablets The proposed method was evaluated in the assay of commercially available tablets containing 50 mg of Enalapril and 5 mg of Felodipine. Six replicate determinations (n=6) were carried out on an accurately weighted amount of the pulverized tablets equivalent to 25 mg of Enalapril and 2.5 mg of amoldipine as amoldipine besylate. The label claim found was to be 47.96-50.21 mg of Enalapril and 4.85-5.02 mg of Felodipine per tablet (table 5).

CONCLUSION

The proposed high-performance liquid chromatographic method has been evaluated over the accuracy, precision and linearity and proved to be more convenient and effective for the quality control and identity of Enalapril and Felodipine in pharmaceutical dosage forms. The measured signals were shown to be precise, accurate and linear over the concentration range tested (0–150%) with a correlation coefficient better than 0.9991. Moreover, the lower solvent consumption along with the short analytical run time of 5.0 minutes leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. Therefore, this HPLC method can be used as a routine sample analysis.

REFERENCES

[1] Prisant LM. *J. Hypertens*., **2002**; 20: S11-S19.
[2] Moser M. Am. *J. Hypertens*., **1998**;11: 69S-72S.
[3] Moser M and Black HR. Am. *J. Hypertens*., **1998**;11: 73S-78S.
[4] Waeber B, Detry JM and Dahlof B. *Am. J. Hypertens*., **1999**;12: 915-920.
[5] Deanfield JE, Detry JM and Sellier P. *J. Am. Coll. Cardiol*. **2002**; 40: 917-925.
[6] Davies RF, Habibi H and Klinke WP. J. Am. Coll. Cardiol., **1995**;25: 619- 625.
[7] Mettimano M, Pichetti F and Fazzari L. *Int. J. Clin. Pract.,* **1995**; 54: 424-428.
[8] Li-Ping X, Fu-Ming S, He S, Chao-Yu M, Yúan-Ying J and Ding-Feng S. *Acta. Pharmacologica. Sinica*, **2005**;26(11): 1303-1308.
[9] Kasture AV and Ramteke M. *Indian J. Pharm. Sci*., **2006**;68(3): 394-396.
[10] Ravishankar S, Nanjan MJ, Vasudevan M, Shaat N and Suresh B. *Indian J. Pharm. Sci*., **1997**;59: 171-173
[11] ICH, Q2B, (**1995**). Validation of analytical procedure: methodology. International Conference on Harmonisation, London.
[12] Menard J. *Blood Press. Suppl*., **1993**; 1: 5-9.