

DEVELOPMENT AND VALIDATION OF A REVERSE PHASE HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SOME PRILLS IN DRUG FORMS

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ABSTRACT

A simple, economical, precise, accurate, and rapid HPLC method has been developed and validated for assay determination of captopril, lisinopril and imidapril simultaneously in their raw material and tablet dosage forms. The chromatographic condition was performed on a mixture of acetonitrile and phosphate buffer (25:75 v/v) ratio. The detection of Prills drugs was carried out at 210 nm with a flow rate of 1.0 ml/min. The retention times for lisinopril, captopril and imidapril were 3.6, 4.4, and 7.4 min respectively. Results of the analysis were validated statistically, and by recovery studies. The proposed method was successfully employed for the estimation of the drug contents in marketed formulation, according to ICH guidelines and found to be suitable for simultaneous determination of Prills.

Keywords: Prills, Captopril, Imidapril, Lisinopril, RP-HPLC, Validation.

INTRODUCTION

Lisinopril, imidapril and captopril are group of drugs that originally synthesized from compounds found in pit viper venom and used primarily for the treatment of hypertension and congestive heart failure [1-2]. They are angiotensin converting enzyme inhibitors (ACEIs). ACEIs lower the blood pressure in hypertensive patients as well as in salt-depleted normotensive patients [3-4]. The blood pressure change is related to pretreatment plasma-renin activity and angiotensin levels. If the patient is hypertensive with high level of plasma-renin activity, the blood pressure will be reduced to greatest level [5-9]. Furthermore, several studies have suggested that ACEI can prevent cataract genesis by scavenging free oxygen radicals [10].

Many studies reported various analytical methods for the estimation of lisinopril, captopril and imidapril in pharmaceutical formulation separately or with other drugs [11-14]. However, there are no methods available to determine the combined mixture of lisinopril, captopril and imidapril. Thus, the present study is focused on a successful attempt to estimate lisinopril, captopril and imidapril by using a single economic, simple, precise and accurate reversed phase HPLC method in pharmaceutical preparation. Such methodology is cost effective in terms of time required for analysis, solvent noise and extraction steps.

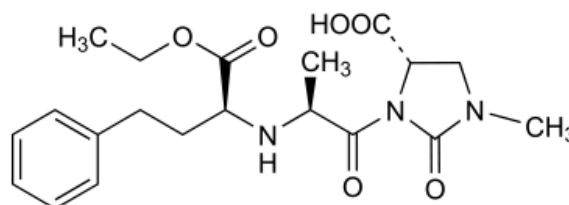


Fig. 1c: Imidapril

MATERIALS AND METHODS

Materials

Pharmaceutical grade lisinopril, captopril and imidapril were supplied by Hikma Pharmaceuticals -Jordan, and were used without further purification. All chemicals and reagents were HPLC grade and analytical grade. acetonitrile of HPLC grade (ACROS), deionized water (Nanopure), potassium dihydrogen phosphate (Scharlau), and phosphoric acid 85% (Merck). Tablets containing captopril 50mg, lisinopril 20 mg, imidapril 10 mg were procured from DAD Pharmaceuticals, Astra-Zeneca, and Hikma Pharmaceuticals, respectively.

Instrumentation and Chromatographic Conditions

A Dionex HPLC auto-sampler system was used and composed of a constant solvent delivery system (P580), 100 µL fixed volume injector (Rheodyne 7125), UV detector (UVD 340S), Autosampler (ASI-100) with Chromeleon Chromatography Management System, BDS Hypersil Phenyl Column (250 x 4.6 mm), and STH 585 Column oven.

Different mobile phases were tested in order to find the best conditions for composition of mobile phase was determined to be buffer: acetonitrile(75:25 v/v) and flow rate was set to 1.0 ml/min.

Method

Selection of Wavelength

Wavelength determination for the drug solution lisinopril, captopril and imidapril was observed by UV-VIS scan (200-550 nm). There absorbance detection is ranged from 200-250 nm. The proper wavelength used was 210 nm.

Buffer preparation

The buffer solution is prepared by weighing 6.84 g of potassium dihydrogen phosphate (KH₂PO₄) and added to 1000 mL of deionized

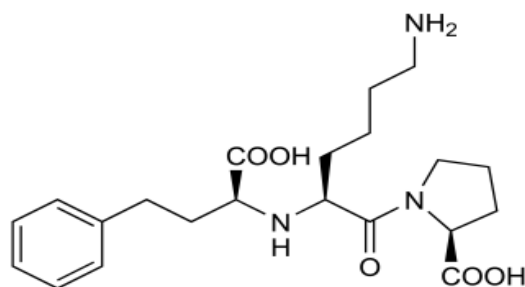


Fig. 1a: Lisinopril

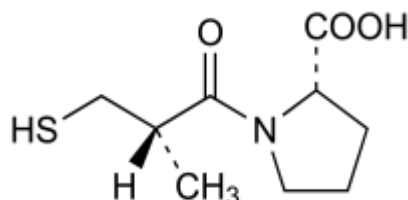


Fig. 1b: Captopril

water (HPLC grade). This weight of KH_2PO_4 was required to make 50 mM buffer.

Mobile phase preparation

The mobile phase preparation is based on the aqueous: organic ratio of buffer: acetonitrile (75:25) with adjusted pH to 3 by using phosphoric acid. Sonication is required to get rid of the air bubbles.

Standard solution preparation

A stock solution of each drug lisinopril, captopril and imidapril was prepared by dissolving of 10.9 mg, 10 mg, and 10.9 mg respectively, in 10 mL of mobile phase solution (buffer: acetonitrile 75%:25%, v:v), aliquot 1 mL of solution and diluted to 10 mL of mobile phase (dilution) to prepare 100 $\mu\text{g}/\text{mL}$.

Sample solution preparation

A stock solution of each drug; lisinopril, captopril and imidapril was prepared by dissolving of 10.9 mg, 10 mg, and 10.9 mg respectively, in 10 mL of mobile phase solution (buffer: acetonitrile, 75%:25%, v:v), aliquot 1 mL of solution and diluted to 10 mL of mobile phase (dilution) to prepare 100 $\mu\text{g}/\text{mL}$.

Placebo solution preparation

The placebo solution is prepared by using 100 mg of these excipient; aerosil 60%, Mg-stearate 5%, glucose 20% and starch 15% and dissolved in mobile phase solution as solvent. This solution is injected to HPLC system

RESULTS AND DISCUSSION

To develop a precise, accurate and suitable RP- HPLC method for the simultaneous estimation of lisinopril, captopril and imidapril, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The proposed HPLC method was validated as per ICH guidelines [16]

Many tests applied to develop a method for all the three drugs with different mobile phase compositions, buffers, pH's, columns and temperatures. All the tests had been applied showed asymmetrical peaks, overlapping, and unusual chromatograms for the drugs separately and in mixture in solution. The method 75%:25% buffer: acetonitrile, 50 mM KH_2PO_4 with pH of 3 was the best one for this group of drugs.

System Precision

The first type of precision test was the system precision. The purpose of this test is to find the degree of agreement between individual test results when the procedure is applied repeatedly to multiple injections. The data obtained showed in Table 1. The data were obtained showing RSD% value below 2% (according to USP), retention time is good for the drugs separation (4-8 min), and no overlapping between peaks obtained from resolution data which indicate precise system.

Table 1: System precision test results

Parameters	Lisinopril	Captopril	Imidapril
Concentration ($\mu\text{g}/\text{mL}$)	100	100	100
Average Area for 10 injections	25.562	21.769	25.725
RSD%	1.190%	1.145%	1.185%
Asymmetry (USP)	1.14	0.96	0.96
Resolution	0	4.33	11.70
Theoretical Plates (USP)	7073	5227	8938
Retention time (min)	3.59	4.49	7.91

Intermediate precision

Table 2 shows the results of the test that obtained by running the samples in two days by using different analysts. In second day, the same chromatographic conditions applied and the concentration

was 100%. Assay% and RSD% values obtained are within range 98%-102% (± 2), which indicate a valid method. These precision tests were applied for the three drugs and the data observed are gave a precise and valid method of analysis.

Table 2: Results of Intermediate precision

Sample #	Assay %		
	Lisinopril	Captopril	Imidapril
1	100.946%	100.877%	100.643%
2	99.146%	99.271%	98.825%
3	98.745%	98.929%	98.441%
4	100.137%	100.303%	99.756%
5	100.699%	100.945%	100.408%
6	100.059%	100.225%	99.652%
Average	99.955%	100.091%	99.620%
RSD%	0.860	0.828	0.865

Linearity

For all the three drugs, $R^2 < 1$ and the calibration curve equation showed a good linearity curve which means that the linearity test is validated (Table 3).

Table 3: Linearity data for lisinopril, captopril, and imidapril.

Material	R ²	Slope	Intercept	Calibration curve equation
Lisinopril	0.993	0.2420	0.4984	$y = 0.242x + 0.498$
Captopril	0.998	0.2070	0.6200	$y = 0.207x + 0.619$
Imidapril	0.999	0.2475	0.5511	$y = 0.247x + 0.551$

Accuracy

The accuracy test was applied in different levels of concentrations for the three drugs in one sample solution with triple injections for each sample (Tables 4-6). The % of recovery equation is:

$$\% \text{ Accuracy} = \left[\frac{\text{recovered amount}}{\text{actual amount}} \times 100 \right]$$

The accepted limits of recovery are 98%-102% according to USP and all observed data are within the required range that indicates good recovery values.

Table 4: Results of accuracy of lisinopril

Concentration %	50%	100%	150%
Area	12.948	25.497	38.135
Assay %	100.621%	99.075%	98.789%
RSD%	0.217%	0.123%	0.070%

Table 5: Results of accuracy of captopril

Concentration %	50%	100%	150%
Average Area	10.267	20.683	31.440
Assay %	98.527%	99.241%	100.569%
RSD%	0.346%	0.090%	0.074%

Table 6: Results of accuracy of imidapril

Concentration %	50%	100%	150%
Average Area	12.984	25.633	38.422
Assay%	100.278%	98.987%	98.915%
RSD%	0.279%	0.149%	0.246%

In addition, figures 2, 3 and 4 indicate the relationship in which the changing in concentration levels will cause changing peak areas (AUC), by increasing the concentration, the AUCs will increase.

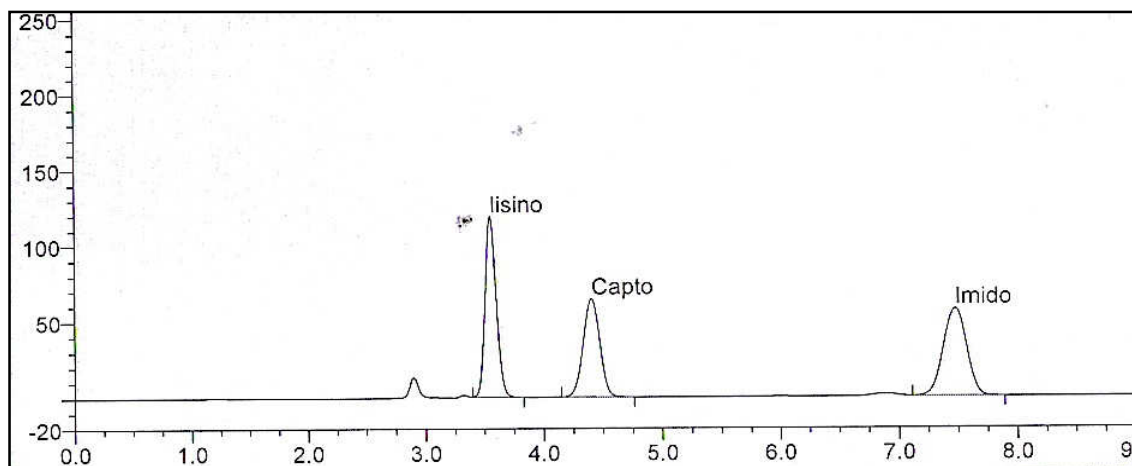


Fig. 2: chromatogram of level 50% of lisinopril, captopril, and imidapril sample

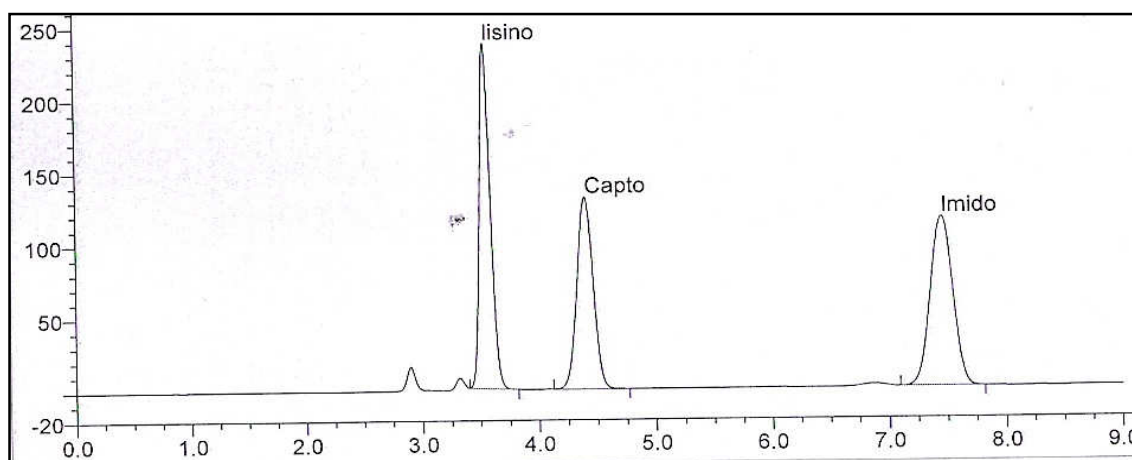


Fig. 3: Chromatogram of level 100% of lisinopril, captopril, and imidapril sample

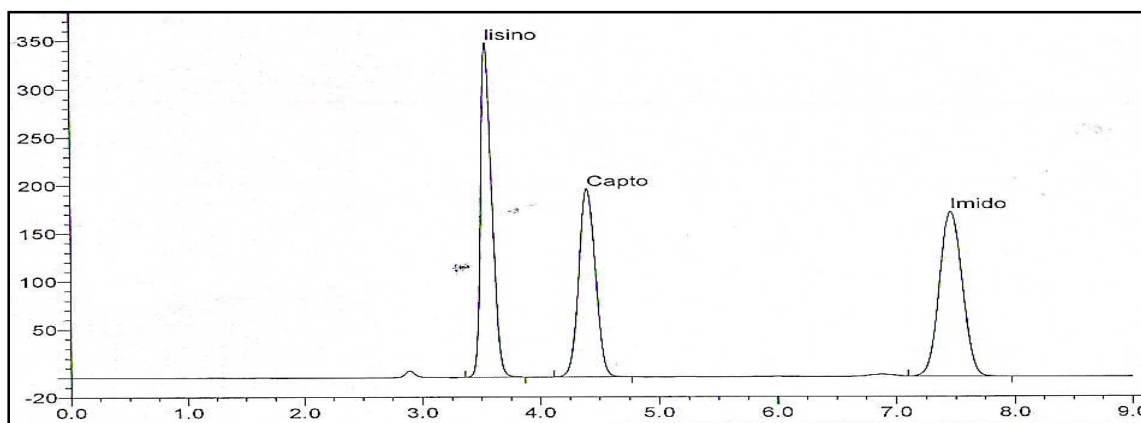


Fig. 4: Chromatogram of level 150% of lisinopril, captopril and imidapril sample

Stability of analytical solution

The stability test was done at different temperatures and times according to ICH guideline. Tables 7, 8, 9 represent the results of stability after 24 hours, while tables 10, 11, 12 showed the results after 48 hrs. The % assay results under all tested conditions are within the accepted USP range 98%-102%. Such results indicate that the drugs are stable under the test conditions.

Robustness

This test is applied to improve the method robustness by make a variation in procedure parameters within certain limit without change in results that obtained. Robustness varies with the

procedure applied. Generally, it is done by varying procedure parameters and observing its effect on the analyte analysis. Robustness was performed using solutions prepared in a similar fashion as system or method precision, the number of replicates (typically 2 or 3), and was evaluated based on system suitability parameters or on recovered amounts, both compared to data generated using the original method. The following changes were done separately: detector wavelength (± 3 nm), pH of mobile phase (± 0.2), mobile phase composition ($\pm 5\%$ - 10%) of acetonitrile volume and temperature (± 3) Celsius. Slight variation in wavelength had been done to the analytical method in order to evaluate and measure the capacity of the method to remain unaffected by small variations (Table 13, 14).

Table 7: Results of stability test of lisinopril after 24 hrs

Time and Temp.	Average of AUCs , Lisinopril (100 ug/mL)	Assay %
Standard solution	25.910	N.A
24 hr at 25 °C	26.034	100.481%
24 hr at 4 °C	25.946	100.138%

Table 8: Results of stability test of captopril after 24 hrs

Time and Temp.	Average of AUC , Captopril (100 ug/mL)	Assay %
Standard solution	22.464	N.A
24 hr at 25°C	22.766	101.347%
24 hrs at 4 °C	22.522	100.258%

Table 9: Results of stability of imidapril after 24 hrs

Time and Temp	Average of AUC, Imidapril (100 ug/mL)	Assay %
Standard solution	26.228	N.A
24 hr at 25°C	26.684	101.738%
24 hrs at 4 °C	26.373	100.55%

Table 10: Results of stability test of lisinopril after 48 hrs

Time and Temp	Average of AUC of Lisinopril (100 ug/mL)	Assay %
Standard solution	25.910	N.A
48 hrs in 25 °C	25.917	100.029%
48 hrs in 4 °C	26.319	101.580%

Table 11: Results of stability test of captopril after 48 hrs

Time and Temp	Average of AUC of Captopril (100 ug/mL)	Assay%
Standard solution	22.464	N.A
48 hrs in 25 °C	22.318	99.352%
48 hrs in 4 °C	22.712	101.104%

Table 12: results of stability test of Imidapril after 48 hrs

Time and Temp	Average of AUC of Imidapril (100 ug/mL)	Assay%
Standard solution	26.228	N.A
48 hrs in 25 °C	26.152	99.708%
48 hrs in 4 °C	26.613	101.467%

Table 13: Results of robustness regarding wavelength (+3)

Parameters	Wavelength (210 nm)			Wavelength (213 nm)		
	Lisinopril	Captopril	Imidapril	Lisinopril	Captopril	Imidapril
Area	24.656	21.959	25.425	22.371	18.663	26.340
RSD%	0.224	0.201	0.262	0.603	0.490	0.442
Theoretical plates	7369	5033	8233	7516	5160	8537
Asymmetry (USP)	1.16	0.97	0.98	1.16	0.97	0.98
Resolution (USP)	0	4.11	10.55	0	4.15	10.71

Table 14: Results of robustness regarding wavelength (-3)

Parameters	Wavelength (210 nm)			Wavelength (207 nm)		
	Lisinopril	Captopril	Imidapril	Lisinopril	Captopril	Imidapril
Area	24.656	21.959	25.425	26.228	26.147	24.866
RSD%	0.224	0.201	0.262	0.303	0.385	0.385
Theoretical plates	7369	5033	8233	7466	5081	8205
Asymmetry (USP)	1.16	0.97	0.98	1.15	0.97	0.97
Resolution (USP)	0	4.11	10.55	0	4.13	10.57

The slight change in wavelength (± 3) gave a slight variation in AUCs of drugs, but the RSD% values are still within the range (± 2) and the resolution values are not changed. The Assay% couldn't be calculated for lisinopril, captopril and imidapril because of the variation in wavelength is not optimum for all the drugs used, by increasing the wavelength 213 nm lisinopril and captopril AUC are decreased but imidapril is increased. Whereas decreasing the wavelength 207 nm

the area of lisinopril and captopril are increased but imidapril's area is decreased.

According to change pH ± 0.2 units, the main pH based in this method is 3 and the results are shown in tables 15, 16 and 17 and figures 5, 6 and 7. These results showed the analytical method is robust for lisinopril captopril and imidapril in variation to pH.

Table 15: Robustness regarding pH = 3

Material	Lisinopril	Captopril	Imidapril
Assay%	100%	100%	100%
RSD%	0.134%	0.150%	0.288%

Table 16: Robustness regarding pH (+ 0.2), pH= 3.2

Material	Lisinopril	Captopril	Imidapril
Assay%	101.001	100.815	99.919
RSD%	0.088	0.227	0.060

Table 17: Robustness regarding to pH (-0.2), pH=2.8

Material	Lisinopril	Captopril	Imidapril
Assay%	101.816	101.168	101.531
RSD%	0.266	0.396	1.350

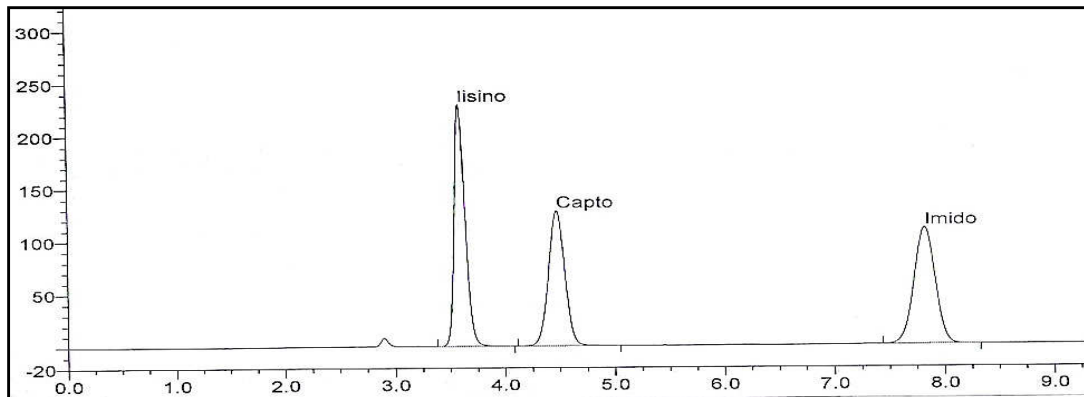


Fig. 5: Chromatogram of lisinopril, captopril, and imidapril mixture at pH= 3

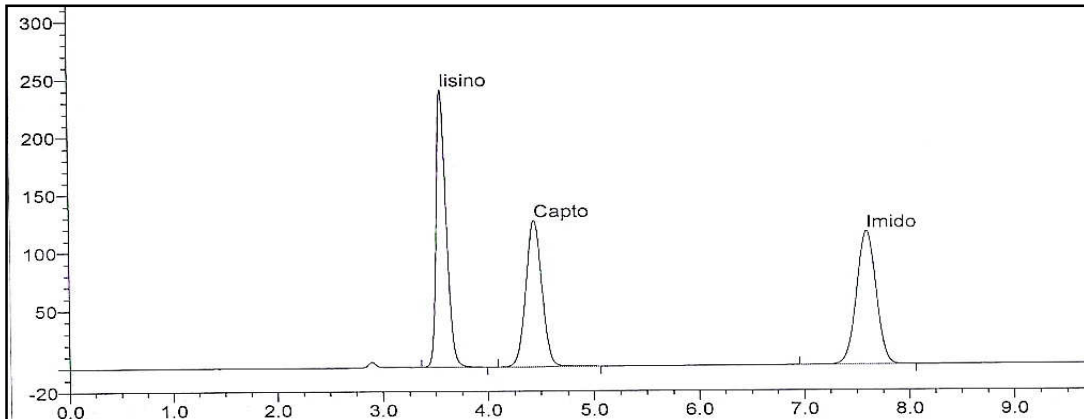


Fig. 6: Chromatogram of lisinopril, captopril, and imidapril mixture at 3.2

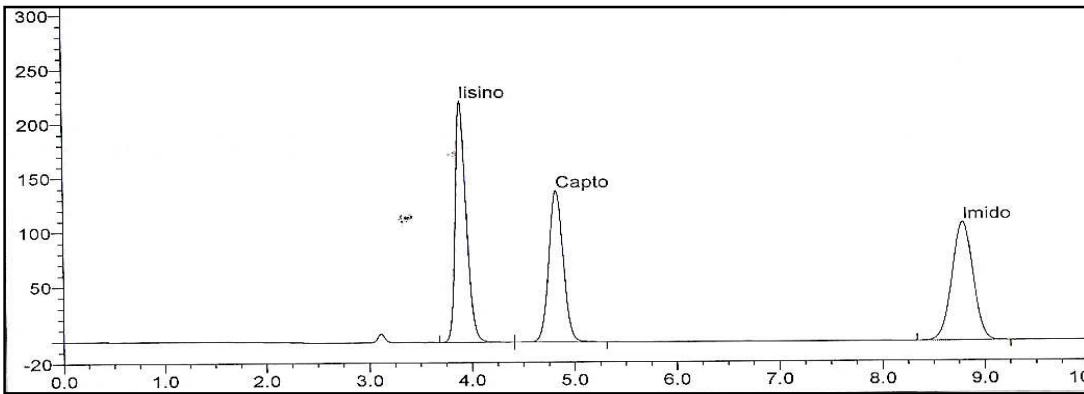


Fig. 7: Chromatogram of lisinopril captopril, imidapril mixture at pH = 2.8

In this study lisinopril is routinely analyzed by RP-HPLC at room temperature can be readily observed splitting peak for lisinopril (Figure 8). Besides, at high temp 50 °C and 50 mM phosphate buffer with acetonitrile (75%/25%, v/v) gave a favorable separation and retention time for lisinopril conformers and sharp peak shape.

Regarding to change in temp in range ($\pm 3^\circ\text{C}$), the method shows robustness in this change (Table 18 &19, Figures 10 &11). There was no change in RSD% and AUCs values by changing in temperature; which indicated a good robustness regarding changes in temperature.

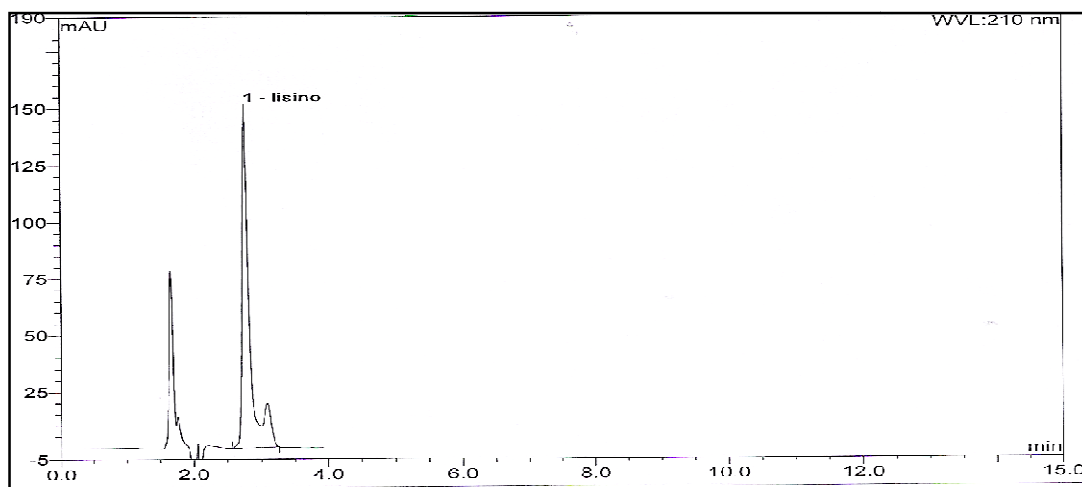


Fig. 8: Splitting peak of lisinopril at room temperature.

Table 18: Robustness regarding temp change (+3)

Parameters	Temp. 50 °C			Temp. 53 °C		
	Lisinopril	Captopril	Imidapril	Lisinopril	Captopril	Imidapril
Area	24.656	21.959	25.425	24.639	21.982	25.414
RSD%	0.224	0.201	0.262	0.301	0.366	0.367
Theoretical plates	7369	5033	8233	7875	5695	7944
Asymmetry	1.16	0.97	0.98	1.13	0.99	0.97
Resolution	0	4.11	10.55	0	4.24	10.51

Table 19: Robustness regarding temperature change (-3)

Parameters	Temp. 50 °C			Temp. 47 °C		
	Lisinopril	Captopril	Imidapril	Lisinopril	Captopril	Imidapril
Area	24.656	21.959	25.425	24.938	22.169	25.658
RSD%	0.224	0.201	0.262	0.321	0.308	0.316
Theoretical plates	7369	5033	8233	7731	4585	8752
Asymmetry	1.16	0.97	0.98	1.17	0.95	0.98
Resolution	0	4.11	10.55	0	4.08	10.73

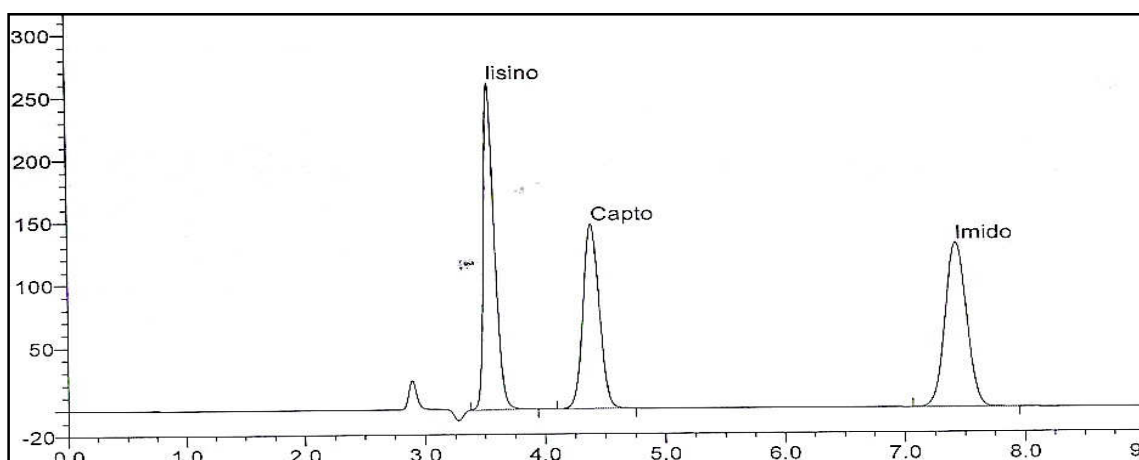


Fig. 9: Chromatogram of lisinopril, captopril, and imidapril mixture in 50 °C (standard).

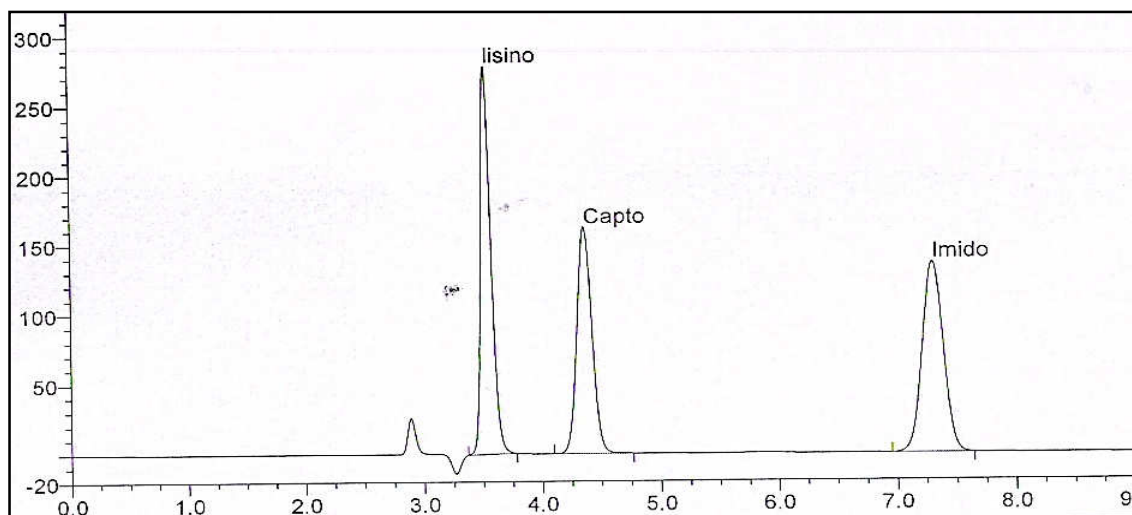


Fig. 10: Chromatogram of lisinopril, captopril, and imidapril mixture in 53 °C.

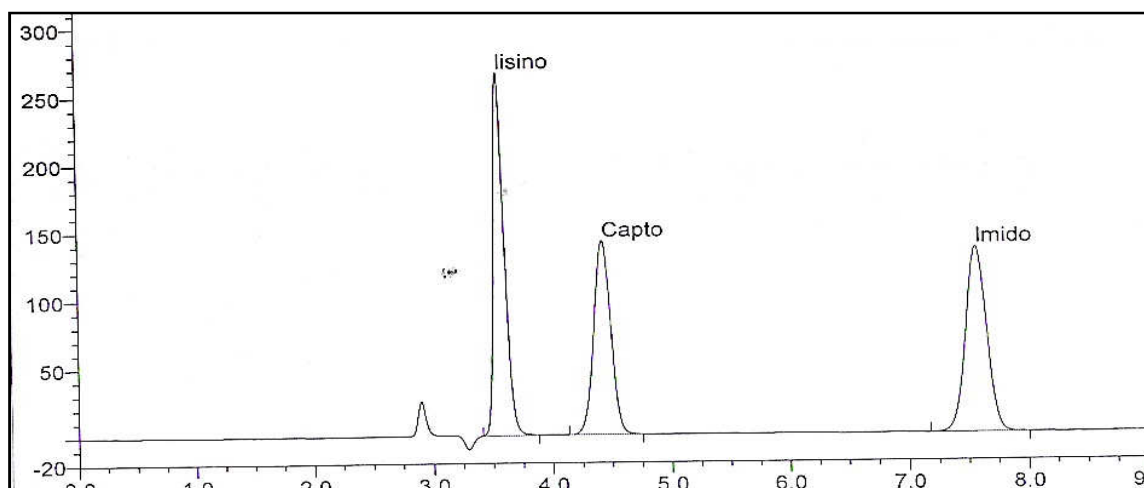


Fig. 11: Chromatogram of lisinopril, captopril, and imidapril mixture in 47 °C

Slight variations in composition of mobile phase have been done to the analytical method to evaluate and measure the capacity of the method to remain unaffected by small variation (Table 20& 21, Figures 12 &13).

Table 20: Robustness regarding organic modified in mobile phase (+5%)

Parameters	Mobile phase 75%-25%			Mobile phase 80%-20%		
	Lisinopril	Captopril	Imidapril	Lisinopril	Captopril	Imidapril
Area	26.113	21.744	24.751	25.934	21.664	24.261
RSD%	0.134%	0.150%	0.288%	0.086	0.138	0.099
Assay %	100%	100%	100%	99.313	99.634	98.018
Theoretical plates	7369	5033	8233	4784	3007	6261
Asymmetry	1.16	0.97	0.98	1.16	0.93	0.91
Resolution	0	4.11	10.55	0	3.82	14.02

Table 21: Robustness regarding organic modified in mobile phase (-5%)

Parameters	Mobile phase 75%-25%			Mobile phase 70%-30%		
	Lisinopril	Captopril	Imidapril	Lisinopril	Captopril	Imidapril
Area	26.113	21.744	24.751	25.793	21.411	24.665
RSD%	0.134%	0.150%	0.288%	0.663	0.201	0.198
Assay%	100%	100%	100%	98.470	98.470	99.651
Theoretical plates	7369	5033	8233	8318	6025	7726
Asymmetry	1.16	0.97	0.98	1.18	1.01	0.96
Resolution	0	4.11	10.55	0	4.01	7.75

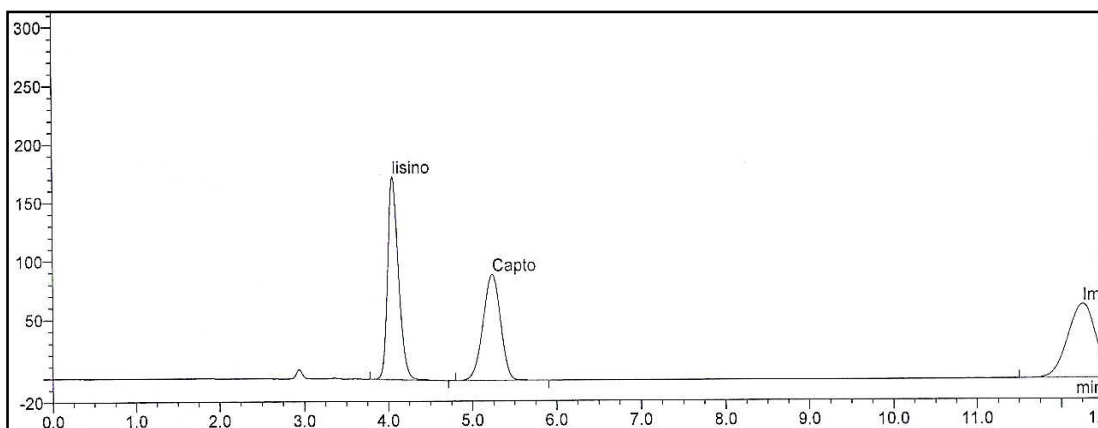


Fig. 12: Chromatogram of Lisinopril, Captopril, Imidapril mixture in mobile phase (+5%).

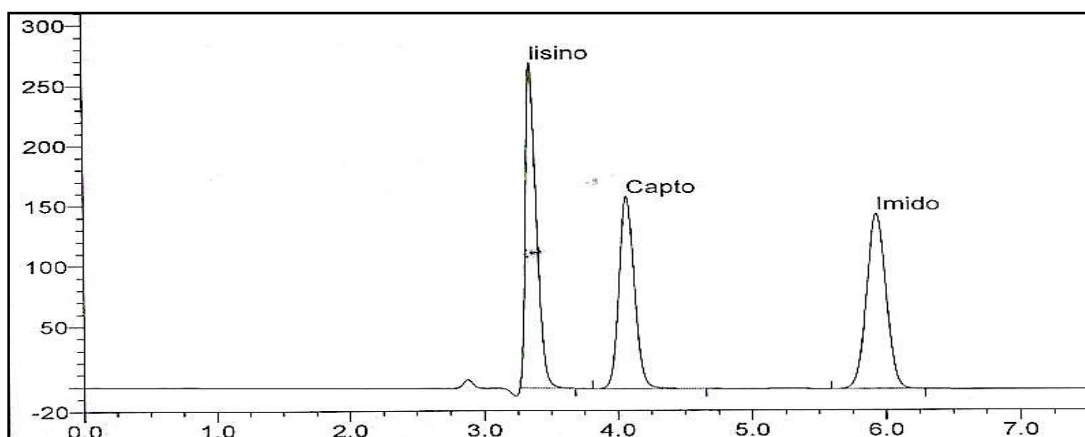


Fig. 13: Chromatogram of Lisinopril, Captopril, and Imidapril mixture in mobile phase (-5%)

These results show that the RSD % < 2% and chromatograms in figures 13 and 14 gave indication that the method is robust with the small variation in mobile phase ratio.

Imidapril is affected by changing the organic solution; decreasing the retention time of imidapril by increasing the organic phase (Acetonitrile, ACN) figure 14:

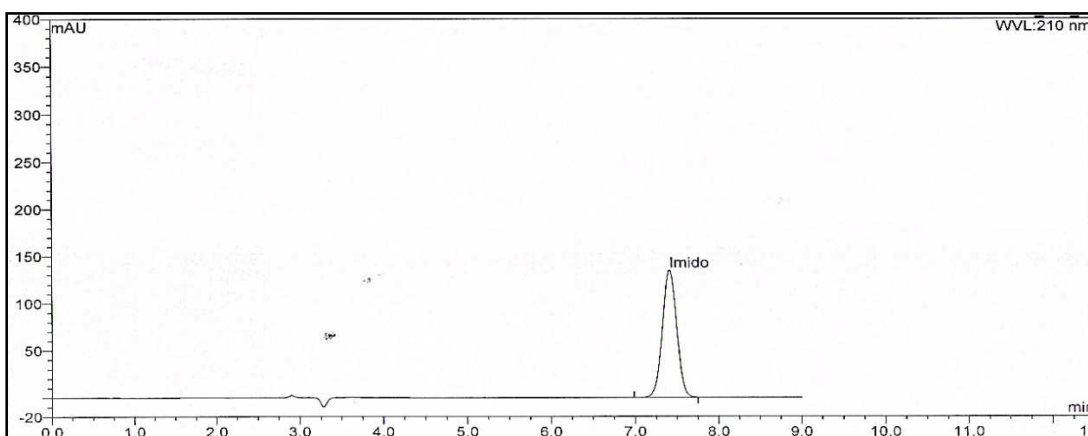


Fig. 14: Imidapril identification chromatogram.

Placebo analysis

A placebo solution prepared in Lab based on the most common and available excipients. These are Aerosil 60%, Mg-stearate 5%, Glucose 20%, and Starch 15%, by weighing 1g in 10 mL of mobile phase as solvent (75%:25%, buffer:ACN, v%:v%). The placebo sample is injected twice in system figure 15.

The solutions were injected into system according to the parameters stated under the developed method. It was found

that there is no interference between the analyte and both the solvent and placebo. The selectivity test also includes the analysis of drugs in the pharmaceutical formulation, comparing the results of analysis between local Jordanian manufactured drugs formulations with some international foreign formulations. Table 22 showed the results of comparing between the local products such as Capocard® tablets for captopril, Tanatril® for imidapril and Zestril® for lisinopril with the active material that we used.

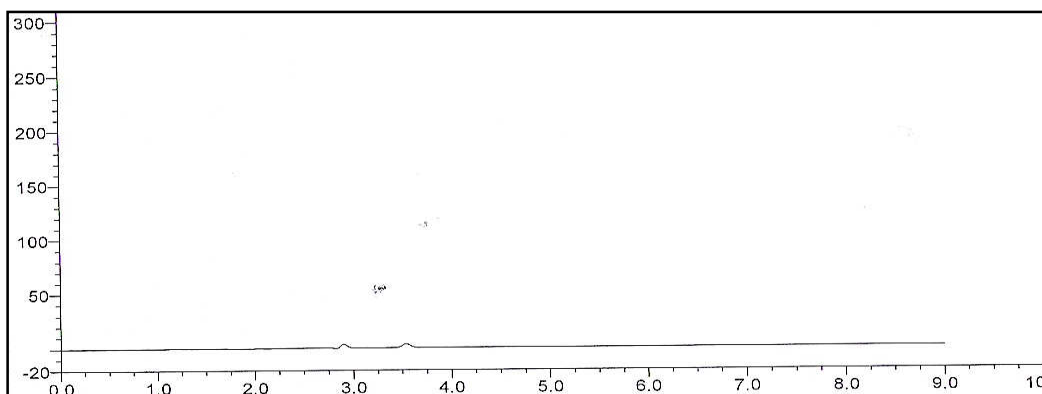


Fig. 15: Placebo chromatogram

Table 22: Recovery % of test and reference formulation

Material	Lisinopril	Captopril	Imidapril
Test formulation	99.495	99.446	99.393
Reference formulation	99.740	99.978	99.753

Force degradation

This test is applied by exposed the active ingredients and finished dosage form, to extreme derivative conditions such as basic (5 mL of

NaOH 0.1M for 1 hr) and acidic (5 mL of HCL 1M for 1 hr) conditions. The results are shown in table 23 and figures 16- 21. The data obtained from this test for lisinopril, captopril, imidapril, are well separated from their degradation products (HCL, NaOH).

Table 23: Peak purity for the standard, active ingredient and placebo solution

Sample name	Purity of Lisinopril	Purity of Captopril	Purity of Imidapril
Standard at normal condition	999.96	999.97	999.98
Sample at normal condition	999.95	999.97	999.98
Standard with 1M HCL	999.95	999.96	999.98
Sample with 1M HCL	999.95	999.96	999.98
Standard with 1M NaOH	999.94	999.95	999.97
Sample with 1M NaOH	999.94	999.95	999.98

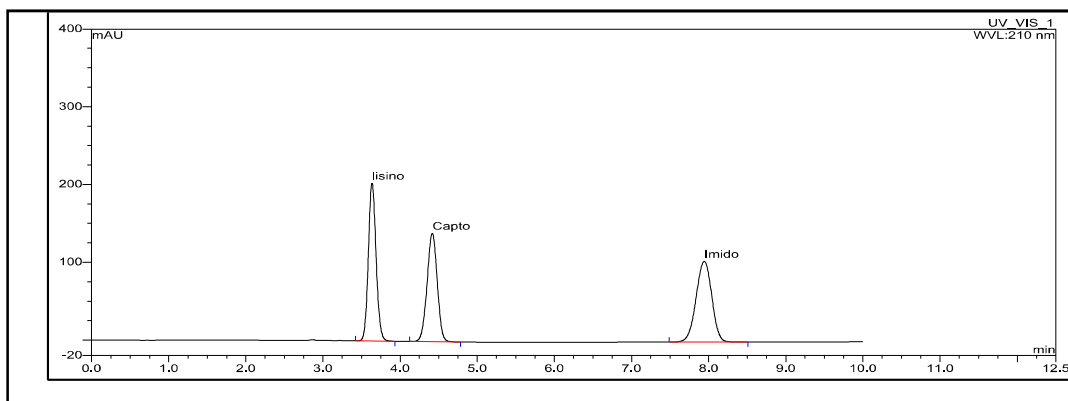


Fig. 16: Force degradation, Normal condition (Standard).

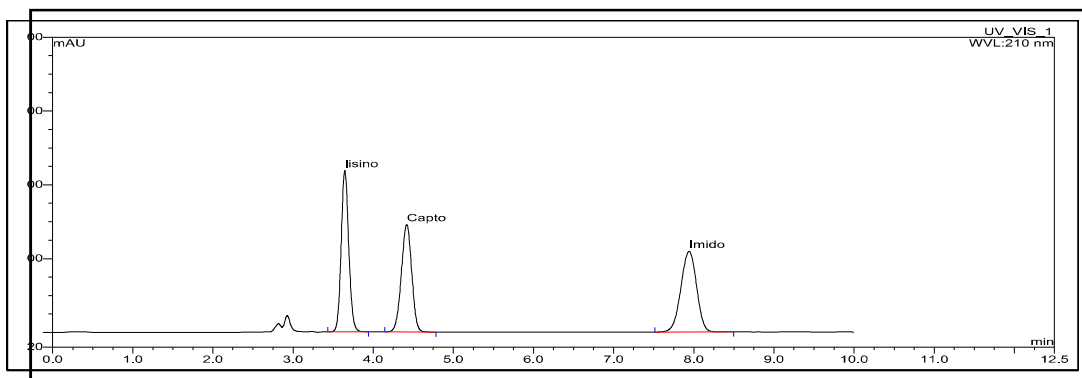


Fig. 17: Force degradation, Basic condition (Standard)

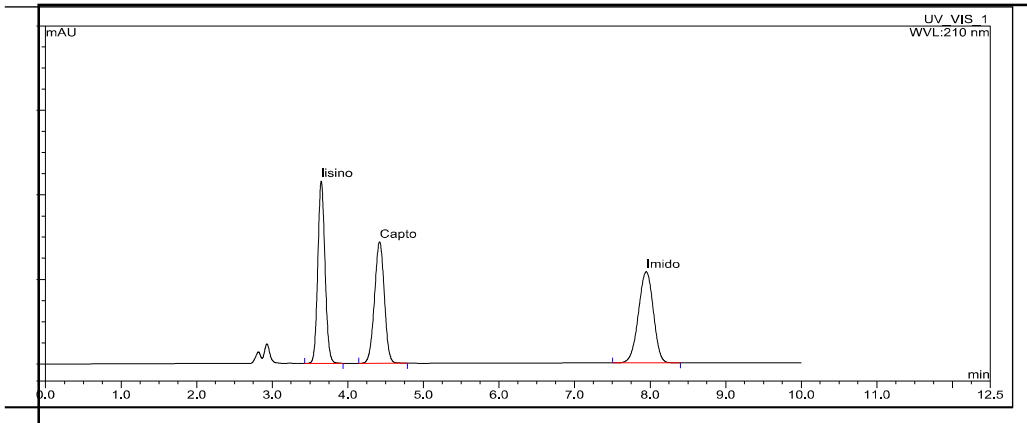


Fig. 18: Force degradation, Acidic condition (Standard).

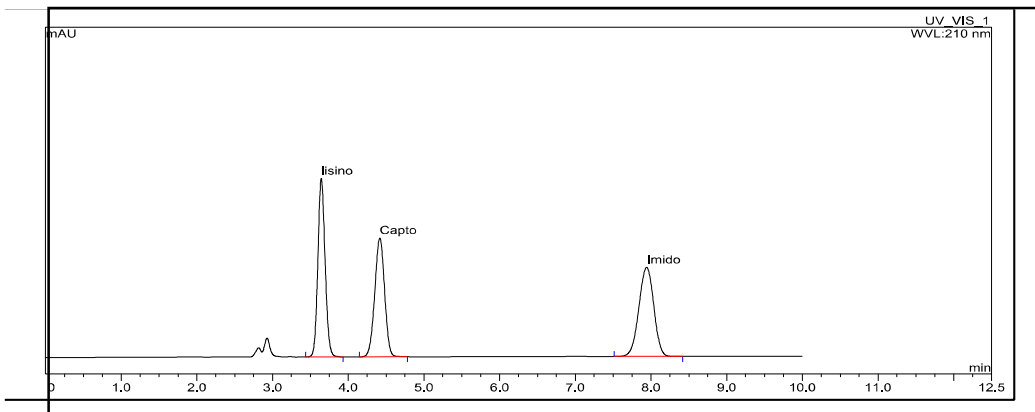


Fig. 19: Force degradation, Acidic condition (Sample)

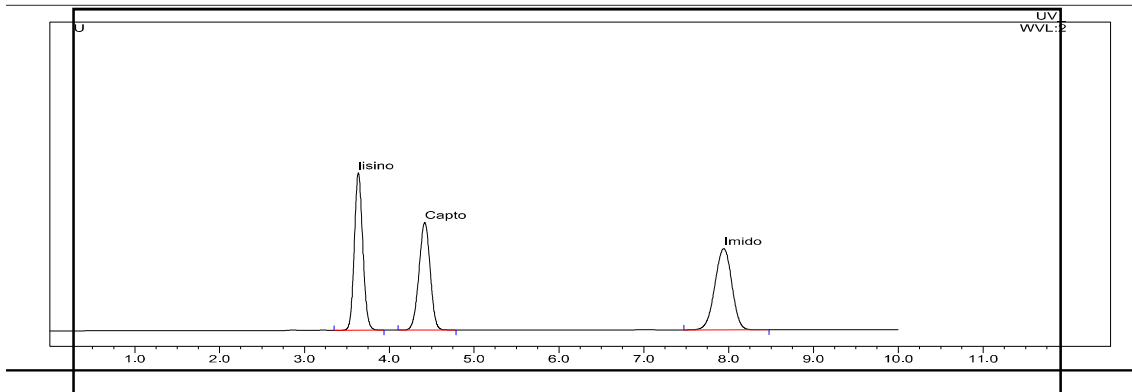


Fig. 20: Force degradation, Normal condition (Sample).

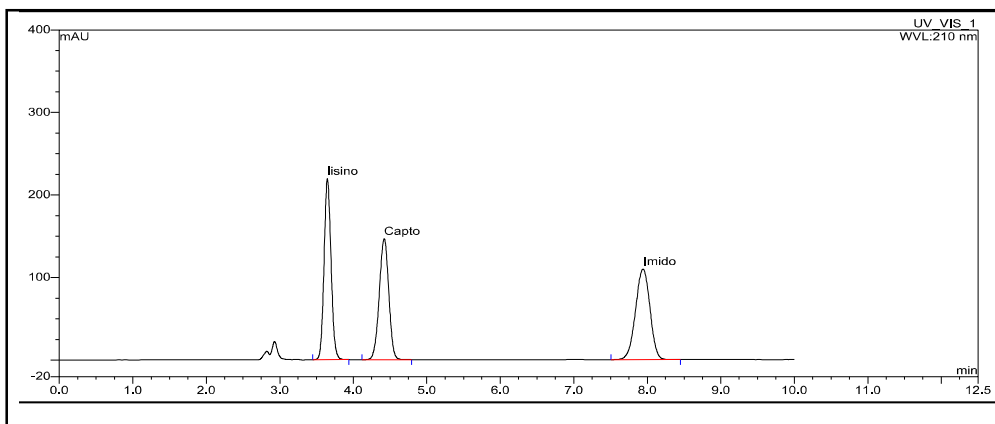


Fig. 21: Force degradation, Basic condition (Sample).

CONCLUSION

A chromatographic experimental method had been applied to develop and validate a single method for the determination of some Prills (Lisinopril Captopril and Imidapril) analysis in pharmaceutical products. The chromatographic conditions were; mobile phase, the column used to give excellent retention, symmetric peak shape, high reproducibility and precise quantitation and resolution. The change in column type and temperature affected the peak separation of lisinopril. Furthermore, increasing the organic phase composition decreased the retention time of the Prills. Finally, the method is economic, accurate, precise and robust with small range of variation in chromatographic conditions and can be used for determination of groups of Prills in pharmaceutical formulation.

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