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RP-HPLC Determination of Lisinopril in Rabbit Plasma

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ABSTRACT

The aim of the current work was to develop a simple HPLC method for the assay of lisinopril in plasma. The HPLC technique was based on the use of reversed phase C8 column. The mobile phase consisted of methanol:distilled water (50:50 V/V) and pH 3.0 was adjusted using phosphoric acid. Captopril was used as an internal standard the method was validated according to ICH guidelines. The flow was adjusted to 1ml/min and the run time lasted 7 minutes. LOD and LOQ were 1.1 & 3.34 µg/ml. The intra- and inter-day precisions (relative standard deviation) were not higher than 0.5% and accuracy (relative error) did not exceed 14.3%. The linearity was observed in the range of 2.5-40 µg/mL with a coefficient of determination of 0.993. The developed method was used to calculate the pharmacokinetic parameters following oral administration of lisinopril to rabbits.

INTRODUCTION

Lisinopril (LSP) is an angiotensin converting enzyme (ACE) inhibitor indicated for the treatment of hypertension and heart failure (Ramesh et al., 2010). It may be used alone as initial therapy or concomitantly with other classes of antihypertensive agents or as adjunctive therapy in the management of heart failure in patients who are not responding adequately to diuretics and digitalis (Peter et al., 1984). ACE inhibitors decrease the production of angiotensin II, increase bradykinin level and reduce sympathetic nervous system activity (Robert et al., 2007). They pose a special advantage in the treatment of patient with diabetes, slowing the development and progression of diabetic glomerulopathy (Goa et al., 1997).

LSP is chemically described as (*S*)-1-[*N*2-(1-carboxy-3-phenylpropyl)-*L*-lysyl]-*L*-proline dihydrate1 (Figure 1) (Sultana et al**.,** 2013). It is only available in the market as oral tablets in spite of its low systemic bioavailability (25%) varying from (6-60%) following oral administration due to intersubject variability (Sudarshan and Agham, 2012).

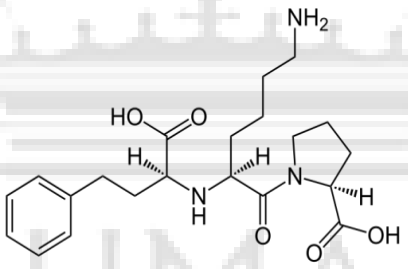

Figure 1. Chemical structure of lisinopril

Due to its slow and incomplete absorption after oral administration with a long elimination half-life reaching 12 hours, the drug has been considered an ideal candidate for the development of new delivery systems which can circumvent the shortcomings of the current strategy. Hence, it deemed necessary to develop a sensitive and accurate method for the determination of drug bioavailability and compare the relative bioavailability's following formulation development and administration.

Various methods have been used for the determination of the drug in bulk and pharmaceutical preparations namely: spectrophotometry (El-Gindy et al., 2001; Paraskos's et al., 2002; El-Yazbi et al., 1999), liquid chromatography–electrospray ionization mass spectrometry (LC–ESI-MS) method (Neng et al., 2008), gas-liquid chromatography (Avadhamulu 1993) capillary electrophoresis and polarography (Hillaert et al., 2001). High-pressure liquid chromatography HPLC methods have been used for the analysis of lisinopril in human plasma/urine using solid-phase extraction with fluorometric detection (El-Emam et al., 2004) or UV detection at 477 nm (Safila, 2014) Liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) method was developed to determine lisinopril in human plasma in concentration range of 1.03–206 ng/mL (Feng et al., 2011). Radioimmunoassay (Hichens et al., 1981; Sun and Mendelsohn, 1991) offered the desired sensitivity (0.2–0.4 ng/mL) but required radiolabelling and anti-lisinopril antiserum which made this method not readily available to all researchers. The tediousness and ease of availability of some of the previously published methods pose difficulty to the accurate determination of drug pharmacokinetics data following administration to animals and humans.

In the present study, the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of LSP in plasma. The method had been applied to determine the main pharmacokinetic parameters following oral administration to a rabbit animal model.

MATERIALS AND METHODS:

a. Materials:

Lisinopril and captopril were kindly supplied as gifts respectively from Astra Zeneca Pharm Company for Pharmaceutical Industries and Squibb for Pharmaceutical Industries, Cairo, Egypt, & orthophosphoric acid were obtained from (Adwic, El-Nasr Pharmaceutical Chemicals Co., Egypt). Methanol HPLC grade (S.D. Fine-chem. Limited, Mumbai, India).

b. Method:

1. Development of HPLC assay method:

Analysis was performed using Waters HPLC 600 controller pump tunable absorbance detector Thermo scientific analytical column with particle size 5µm×250mm ODS hypersil C8 was used. Mobile phase was prepared by mixing methanol: dist. water pH3.0 adjusted using phosphoric

acid. The flow rate was adjusted at 1mL/min and the UV detector was set at a wavelength of 218nm.

Method validation:

1.1. Selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample.

1.2. Linearity and range:

The linearity was checked from constructed calibration curves (Sottanii et al., 2009). Briefly, plasma was spiked with known drug concentrations, then a fixed amount of captopril (10 µg) was added to 0.4ml of plasma and the volume was completed with methanol to 1ml. The final drug concentrations prepared by serial dilution were 2.5, 5, 7.5, 15, 20, 25, 30, 35 & 40 µg/ml. The samples were then centrifuged at 7500 rpm for 15 min, the supernatant was filtered by 0.22µm syringe filter evaporated till dryness then reconstituted to 1000µl in the mobile phase. A sample (20µl) was injected and the peak area ratio of LSP relative to IS was computed. Linearity was determined by calculating the regression line using a mathematical treatment of the peak area ratios *vs.* LSP concentrations in plasma.

1.3. Recovery

The recovery of LSP from rabbit plasma was assessed by adding a known amount of LSP and a fixed amount of internal standard to blank rabbit plasma to give concentrations of 7.5, 30 &40 µg/ml, respectively. then completed to 1mL with methanol centrifugation , evaporation of supernatant and reconstitution with mobile phase was done as mentioned before Recovery percentage was calculated by comparing the peak areas obtained from spiked plasma with those from injected pure standards.

1.4. Precision and accuracy:

Precision and accuracy were assessed by repeated analysis of plasma extracted samples containing different concentrations of LSP on separate occasions in the same day (intra-day) and three different days (inter-day) (Gonzalez et al., 2010; Gonzalez et al., 2011).

Accuracy is a measure of the closeness of test results obtained by a method to the theoretical value. Accuracy was determined by using the following equation.

Precision is a measure of the reproducibility of the whole analytical method (including sampling, sample preparation and analysis) under normal operating circumstances. Precision was determined by repeating the method of assay 6 times and was then expressed as coefficient of variation (CV%).

$$\text{Accuracy} = \frac{(\text{theoretical conc.} - \text{measured})}{\text{theoretical conc.}} * 100$$

1.5. Limit of Detection (LOD)

This is the lowest concentration in a sample that can be detected but not necessarily quantitated, under the stated experimental conditions. The limit of detection is generally quoted as the concentration yielding a signal-to-noise ratio (height of the peak corresponding to the component/ absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution) of 2:1 or 3:1. The detection limit (DL) may also be expressed as (Suresh et al., 2014):

$$\boldsymbol{LOD = 3.3\ SD/\ S}$$

Where ***SD*** = the standard deviation of the response and ***S*** = the slope of the calibration curve.

1.6.Limit of Quantitation (LOQ)

This is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. It is quoted as the concentration yielding a signal-to-noise ratio of 10:1. The quantitation limit (QL) may also be expressed as (Suresh et al., 2014):

$$LOQ = 10\ SD/\ S$$

Where ***SD*** = the standard deviation of the response and ***S*** = the slope of the calibration curve.

2.Pharmacokinetic study:

Male New Zealand rabbits (n=3), weighing 2-3 Kg were used in this study. The rabbits were fasted 12 hrs before and during administration only water was allowed. The experimental procedures conformed to the Ethics Committee of Faculty of Pharmacy, Ain Shams University on the use of animals.

2.1 Drug administration to animals:

The market product was grinded and the required animal dose (0.5 mg/Kg) was calculated, weighed and suspended in distilled water (Sudarshan and Agham,2012). The dispersion was then administered to rabbits orally (n=3).

2.2. Collection of blood samples:

Following oral administration, blood samples were drawn from retro-orbital plexus of rabbits using fine capillary at 0, 0.5, 1, 2, 4, 6, 8, 12, 24 and 36 hrs. Samples were collected in screw capped sterile heparinized centrifuge tubes. Plasma was separated from each sample by centrifugation at 4500 rpm for 5 min. Labeled plasma samples were stored in a glass vial at -20ºC until being analyzed.

2.3.Sample preparation:

Plasma was extracted using protein precipitation using methanol. Briefly, 0.4 ml of Plasma samples were mixed with 10µl of (0.1mg/ml) captopril in methanol as internal standard) and the volume was made up to 1ml with methanol for protein precipitation and extraction of LSP (Sudarshan and Agham,2012). The mixture was vortexed for 5 min, then centrifuged at 7500 rpm for 15 min and finally filtered through a 0.22 µm membrane filter. The sample was then evaporated till dryness then reconstituted in 100 µl mobile phase and 20 µl was injected into the HPLC apparatus for drug content quantification.

2.4.Determination of main pharmacokinetic parameters:

The mean concentrations of LSP in plasma were plotted against the peak area ratio of plasma. The observed peak plasma concentrations (C_{max}) and the time to reach (t_{max}) were derived directly from the plasma concentration data. The area under LSP concentration-time curve (AUC_{0-24}) and (AUC_{0-t}) were calculated by the trapezoidal method with extrapolation to infinity where t is the last measured concentration. The time to reach half concentration in plasma, ($t_{1/2}$) and the elimination rate constant(K_{el}) was calculated from the negative slope of the log-linear termination portion of the plasma concentration- time curve were calculated (Sagirli 2004).

RESULTS AND DISCUSSION

The primary target of this study was to develop a simple and reproducible method for the quantitative determination of LSP in plasma.

Validation of HPLC method

1.1.Selectivity

Figure (2) shows representative chromatograms of (a) blank plasma (b) plasma spiked with 15µg/mL lisinopril (c) plasma sample withdrawn from rabbit after 4h of oral drug administration. Using the described chromatographic conditions, LSP and captopril (IS) were well separated with a mean retention time of 2.4 and 4.8 min respectively. LSP peak was sharp and symmetric with good baseline. No tailing or peak splitting was seen thus allowing accurate peak area calculation. Applying these conditions, the total run time lasted 7min.

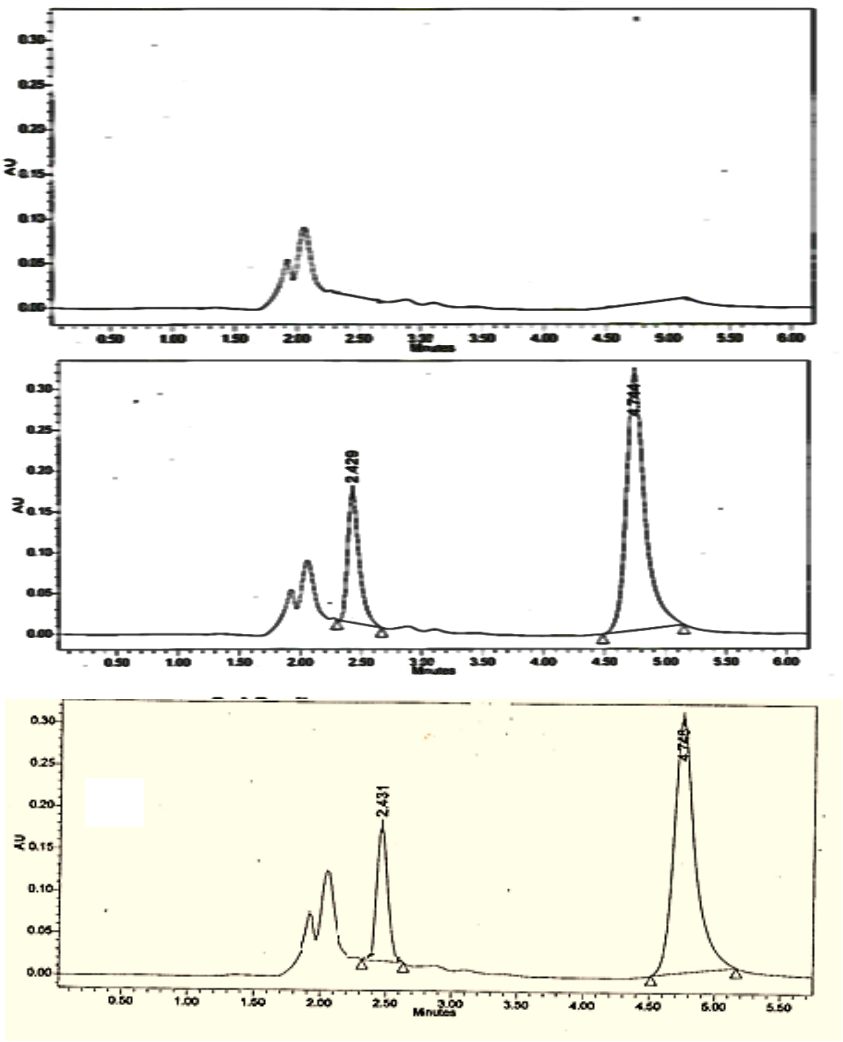

Figure (2): Representative HPLC chromatogram of (a) blank plasma (B) spiked plasma with LSP & captopril (c) sample withdrawn from rabbit after 4 h of oral drug administration.

1.2. Linearity and Range

The standard calibration plots obtained for LSP in samples of rabbit plasma showed a linear relationship between the spiked concentrations in rabbit plasma in the range of 2.5-40 µg/mL and their respective peak areas. The linear regression analysis of the standard calibration plots was **y=0.0401x - 0.0153** where y and x are peak area and LSP concentration respectively. The correlation coefficient ($\mathbf{R^2}$) value was 0.993 (Figure 3).

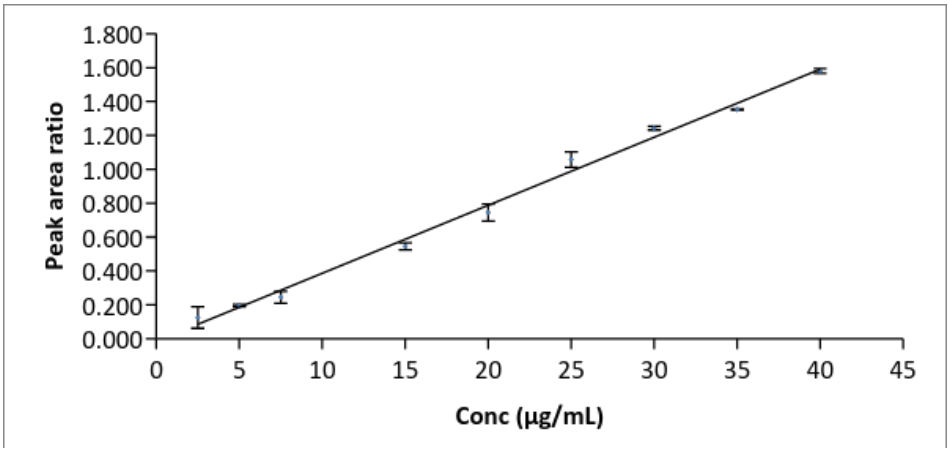

Figure (3): Calibration curve of LSP in rabbit plasma using captopril as an internal standard.

1.3. Recovery:

Different preliminary trials were proceeded to select the best chromatographic conditions. The best recovery for LSP and captopril (IS) from rabbit plasma was obtained using mixture of methanol and distilled water with pH adjusted to 3 (50:50% v/v). The mean recovery percent of LSP was 98.8 (Table 1).

Table (1): Recovery of LSP from spiked rabbit plasma

Spiked conc. (µg/mL)	Recovery %	CV %
7.5	98.04	1.39
30	98.64	0.96
40	99.74	0.18

1.4. Limit of Detection (LOD):

The limit of detection (LOD), corresponding to the lowest concentration that produces a peak height approximately double the baseline noise, is 1.1 µg/mL for plasma. The UV detector was set at wavelength 218 nm to minimize noise effect of plasma.

1.5. Limit of Quantitation (LOQ):

The limit of quantification (LOQ) for plasma 3.34 µg/mL as it was the lowest tested concentration at which both accuracy and precision were within the proposed criteria. Such a limit is adequate for routine therapeutic drug monitoring. However, given the high sensitivity of this assay, achieving a lower limit of quantification seems possible.so this method can offer accurate easy and fast method for monitoring lisinopril in a biological fluid.

1.6. Accuracy and Precision

Table (2) and (3) show the results of intra- and inter-day accuracy and precision in rabbit plasma. The intra-day accuracy was ranged from 0.725 to 14.3with a respective precision of 0.46 to 0.052 expressed as CV%, while the inter-day accuracy had a range of 1.35 to 11.8 with respective CV% of 0.069 to 0.276 denoting sufficient precision .

Table (2): Intra-day accuracy and precision for LSP in spiked rabbit plasma.

Spiked conc.	Mean conc. ± SD	Accuracy (%)	CV (%)
7.5	6.421 ± 0.028	14.3	0.461
30	32.807 ± 0.045	9.3	0.140
40	40.289 ± 0.021	0.7	0.052

Table (3): Inter-day accuracy and precision for LSP in spiked rabbit plasma.

Spiked conc.	Mean conc ± SD	Accuracy (%)	CV (%)
7.5	6.623 ± 0.014	11.69	0.213
30	33.550 ± 0.095	11.8	0.276
40	40.538 ± 0.028	1.35	0.069

Animal study:

To check the applicability of the method, it was used to determine the concentration of the drug following oral administration of a marketed formulation to rabbits in a dose of 0.5mg/mL (Sudarshan and Agham,2012). Figure (4) shows the obtained plasma concentration *vs* time curve. The generated profile was used for the determination of the pharmacokinetic parameters presented in Table (4). As could be seen the maximum plasma concentration was 2.299 µg/mL with a low SD of 0.035 µg/mL among different animals and with a half-life of 19.9 h. The method could thus be used to compare the bioavailability from different formulations by comparing the obtained area under the curves.

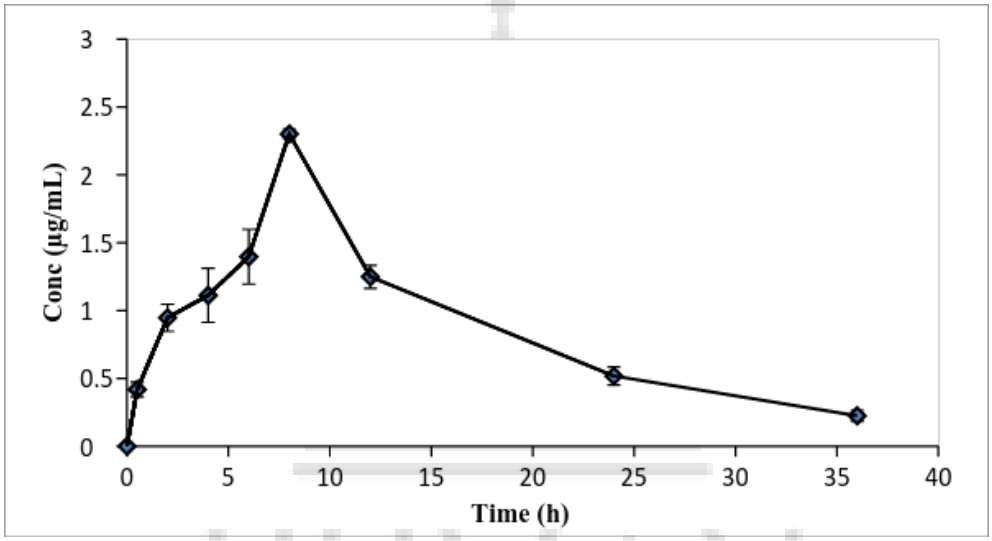

Figure (4): Mean plasma concentration of LSP following single oral dose of commercially marketed available tablets.

Pharmacokinetic parameters:

Table (4): Pharmacokinetic parameters of LSP following a single dose administration of 0.5mg/kg of commercially available oral tablets.

Parameter	Calculated value following oral administration
C_{max} (µg/mL)	2.299 ± 0.035
t_{max} (h)	8.000 ± 0.000
$t_{½}$ (h)	19.930 ± 0.120
AUC_{0-24} (µg.h/mL)	27.300 ± 1.110
AUC_{0-36} (µg.h/mL)	31.750 ± 1.750
K_{el}	0.0347 ± 0.030

CONCLUSION:

In this work, we developed a simple chromatographic HPLC method for the assay of LSP in plasma. The developed method was validated and was applied to determine the plasma concentration following oral administration of LSP market tablet to rabbit. Due to its simplicity and accuracy, the method could be used during product development selection or even during clinical trials.

REFERENCES

1. Avadhamulu AB, Pantulu AR (1993) Gas liquid chromatographic estimation of lisinopril in its pharmaceutical dosage forms. Indian Drugs30: 646-649.
2. El-Emam AA, Hansen SH, Moustafa MA, El-Ashry SM, El-Sherbiny DT (2004) Determination of lisinopril in dosage forms and spiked human plasma through derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) followed by spectrophotometry or HPLC with fluorimetric detection. J Pharm Biomed Anal 34: 35-44.
3. El-Gindy A, Ashour A, Abdel-Fattah L, Shabana MM (2001) Spectrophotometric, septrofluorimetric and LC determination of lisinopril. J Pharm Biomed Anal 25: 913-922.
4. El-Yazbi FA, Abdine HH, Shaalan RA (1999) Spectrophotometric and spectrofluorometric methods for the assay of lisinopril in single and multicomponent pharmaceutical dosage forms. J Pharm Biomed Anal 19: 819-827.
5. Feng Qin, Dan Wang, Shuyan Yang, Lijuan Jing, Zhili Xiong and Famei Li 2011." Quantitative determination of lisinopril in human plasma by high-performance liquid chromatography–tandem mass spectrometry and its application in a pharmacokinetic study." Biomedical. Chromatogr. 26: 691–696.
6. Goa, Karen L.; Haria, Malini; Wilde, Michelle I 1997." Lisinopril: A Review of its Pharmacology and Use in the Management of the Complications of Diabetes Mellitus." Drugs. 53(6):1081-1105.

7. Gonzalez, O., R. M. Alonso, et al. (2011). "Development of an LC-MS/MS method for the quantification of 55 compounds prescribed in compined cardiovascular therapy." J Chromatogr B Analyt Technol Biomed Life Sci **879**(3-4):243-252.
8. Gonzalez, O., G. Iriarte, et al (2010)"LC-MS/MS method for the determination of several drugs used in combined cardiovascular therapy in human plasma." J Chromatogr B Analyt Technol Biomed Life Sci **878(**28): 2685-2692.
9. Hichens M, Hand EL and Mulcahy WS.1981." Radioimmunoassay for angiotensin converting enzyme inhibitors." Ligand Quartery 4: 43.
10. Hillaert S, De Grauwe K, Van den Bossche W (2001) Simultaneous determination of hydrochlorothiazide and several inhibitors of angiotensin converting enzyme by capillary electrophoresis. J Chromatogr A 924: 439-449.
11. Peter C. Gazes, M.D., Michael E. Assey, M.D.1984." The management of congestive heart failure." Current Problems in Cardiology 11(8):1-70.
12. Neng Zhou, Yi-zeng Liang, Ben-mei Chen, PingWang, Xian Chen, and Feng-ping Liu 2008." Development and Validation of LC–MS Method for the Determination of Lisinopril in Human Plasma and its Application in a Bioequivalence Study." Journal of Chromatographic Science 46:848-853.
13. Paraskos's G, Atta-Politou J, Koupparis M (2002) Spectrophotometric determination of lisinopril in tablets using 1-fluoro-2,4-dinitrobenzene reagent. J Pharm Biomed Anal 29: 865-872.
14. Ramesh Gannu, Vamshi Vishnu Yamsani, Chinna Reddy Palem, Shravan Kumar Yamsani & Madhusudan Rao Yamsani2010" Iontophoretic delivery of lisinopril: Optimization of process variables by Box-Behnken statistical design." Journal Pharmaceutical Development and Technology 15(2):169-177.
15. Robert A. Augustyniak, Maria Maliszewska-Scislo, Haiping Chen, John Fallucca and Noreen F. Rossi2007" Acute angiotensin-converting enzyme inhibition evokes bradykinin-induced sympathetic activation in diabetic rats." NIH Public Access 293(6): 2260–2266.
16. Safila Naveed 2014."Analytical Determination of Lisinopril Using UV Spectrophotometer and HPLC: An Overview." Modern Chemistry & Applications3(2):137-140.
17. Sagirli O, Ersoy L (2004) An HPLC method for the determination of lisinopril in human plasma and urine with fluorescence detection. J Chromatogr B Ana
18. Sottani, C. ,E. Leoni, et al. (2009). "Validation of an LC-MS/MS method for determination of epirubicin in human serum of patients undergoing drug eluting microsphere-transarterial chemoembolization (DEM-TACE)." J Chromatogr B Analyt Technol Biomed Life Sci **877**(29): 3543-3548.
19. Sudarshan K. Singh, Agham A. Sameer *2012" Development and characterization of sublingual tablet of Lisinopril." Asian Pacific Journal of Tropical Biomedicine 3(2): 1711-1719.
20. Sultana N, Naveed S and Arayne MS 2013." Direct Determination of Four ACE-Inhibitors Lisinopril, Enalapril, Captopril "chromatography separation technique 4(4):179-184.
21. Sun Y and Mendelsohn FA. 1991."Angiotensin converting enzyme inhibition in heart, kidney, and serum studied ex vivo after administration of zofenopril, captopril, and lisinopril." Journal of Cardiovascular Pharmacology. 18: 478–486.
22. Suresh Babu VV, Sudhakar V and Murthy TEGK 2014" Validated HPLC Method for Determining Related Substances in Compatibility Studies and Novel Extended Release Formulation for Ranolazine." Chromatography Separation Techniques 5(1):209-216.