Development of RP-HPLC method for the determination of lisinopril in human saliva

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ABSTRACT

Background: Reported HPLC methods for determination of lisinopril in plasma are usually tedious requiring several extraction steps necessitating the need for new methods that address these difficulties.

Objectives: This study was aimed at developing and validating a simple and reproducible RP-HPLC method for the determination of lisinopril in human saliva.

Methods: A blank saliva (2 mL) was spiked with 2 mL of a solution (12.5 µg mL^{-1}) of lisinopril followed by 1 mL of a solution (0.05 µg mL^{-1}) of caffeine as internal standard (IS). The mixture was vortex mixed and centrifuged (3000 rpm) for 10 minutes. A portion (0.5 mL) of the resultant clear solution was injected into the HPLC (Agilent model no: 1260 infinity). A chromatogram was obtained which resolved lisinopril from caffeine (IS). The isocratic chromatographic separation was achieved using Chemsl ODS® C18 (200 mm × 4.6 id) analytical column. The optimized chromatographic conditions included a mobile phase of methanol-water (80:20 v/v) containing 0.1 % orthophosphoric acid, isocratic elusion mode, an injection volume of 10 µL, flow rate of 1 mL min^{-1}, a temperature of 35 °C and detection wavelength of 218 nm. A calibration curve of lisinopril reference standard at concentrations range of 1.0 to 50.0 µg mL^{-1} was prepared by plotting the peak height ratios of lisinopril:IS against their corresponding concentrations. The developed method was validated with respect to precision, accuracy, percentage recovery, limit of detection (LOD) and limit of quantitation (LOQ) according to international conference on harmonization (ICH) guidelines.

Results: The chromatographic separation was achieved in 4 minutes with lisinopril and caffeine having retention times of 1.7 and 2.6 minutes respectively. Calibration curve was linear (r = 0.998) within the range of above concentrations. The precision (both inter and intraday) of the method was <1 % RSD, while the accuracy expressed as percentage relative error (% Er) and % recovery were 1.20 and 99.89 % respectively. The LOD and LOQ of the method were 0.15 and 0.46 ng mL^{-1} respectively. All the analytical parameters were within the normal ranges, thus reflecting good recovery and sensitivity of the method.

Conclusion: The results of this study suggest that the developed method can be employed for accurate determination of lisinopril in human saliva.

Keywords: Lisinopril, RP-HPLC, saliva, isocratic elution.

Développement de la méthode RP-HPLC pour la détermination du lisinopril dans la salive humaine

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RESUME

Contexte : Les méthodes HPLC signalées pour la détermination du lisinopril dans le plasma sont généralement fastidieuses et nécessitent plusieurs étapes d'extraction nécessitant de nouvelles méthodes qui répondent à ces difficultés.

Objectifs : Cette étude visait à développer et valider une méthode RP-HPLC simple et reproductible pour la détermination du lisinopril dans la salive humaine.

Méthodes : Une salive vierge (2 mL) a été enrichie d'une solution de 2 mL (12,5 µg mL^{-1}) de lisinopril suivi d'une solution de 1 mL (0,05 µg mL^{-1}) de caféine comme norme interne (IS). Le mélange a été fait avec du vortex et centrifugé (3000 tr/min) pendant 10 minutes. Une portion (0,5 mL) de la solution claire résultante a été injectée dans la HPLC (modèle Agilent n °: 1260 infinity). Un chromatogramme a été obtenu qui a résolu le lisinopril à partir de la caféine (IS). La séparation chromatographique isocratique a été réalisée à l'aide d'une colonne analytique Chemsl ODS® C18 (200 mm x 4,6 id). Les conditions chromatographiques optimisées comprenaient une phase mobile de méthanol-eau (80:20 v/v) contenant 0,1% d'acide orthophosphorique, un mode élusion isocratique, un volume d'injection de 10 µL, un débit de 1 mL min^{-1}, une température de 35 ° C et longueur d'onde de détection de 218 nm. Une courbe d'étalonnage de la norme de référence de lisinopril à des concentrations allant de 1,0 à 50,0 µg mL^{-1} a été préparée en traçant les rapports de hauteur maximale de lisinopril:IS par rapport à leurs concentrations correspondantes. La méthode développée a été validée en ce qui concerne la précision, l'exactitude, le pourcentage de récupération, la limite de détection (LOD) et la limite de quantification (LOQ) conformément aux directives du conseil international d'harmonisation (CIH).

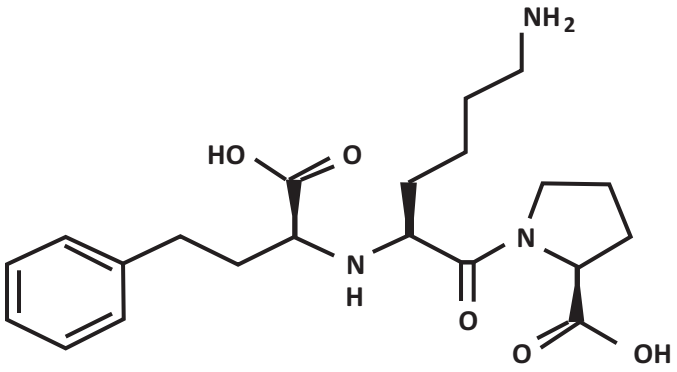
Résultats : La séparation chromatographique a été réalisée en 4 minutes avec du lisinopril et de la caféine ayant des temps de rétention de 1,7 et 2,6 minutes respectivement. La courbe d'étalonnage était linéaire (r= 0,998) dans la fourchette des concentrations ci-dessus. La précision (à la fois inter et intra-journalière) de la méthode était <1% RSD, tandis que la précision exprimée en pourcentage d'erreur relative (% Er) et en % de récupération était de 1,20 et 99,89% respectivement. La LOD et la LOQ de la méthode étaient respectivement de 0,15 et 0,46 ng mL-1. Tous les paramètres analytiques se situaient dans les fourchettes normales, reflétant ainsi une bonne récupération et une bonne sensibilité de la méthode.

Conclusion : Les résultats de cette étude suggèrent que la méthode développée peut être utilisée pour une détermination précise du lisinopril dans la salive humaine.

Mots-clés : lisinopril, RP-HPLC, salive, élution isocratique.

INTRODUCTION

Lisinopril (Figure 1) is chemically defined as (2S)-1-[(2S)-6-Amino-2-[[(1S)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid dehydrate. It is an antihypertensive drug belonging to the angiotensin converting enzyme inhibitors (ACEIs) available in tablet dosage form. It is a white to almost white crystalline powder that is soluble in water, sparingly soluble in methanol, practically insoluble in acetone, chloroform, acetonitrile and in anhydrous ethanol.[1] Lisinopril has pKa value of 3.85 which makes it a stronger basic drug compared to other ACEIs. The lipid solubility of the unionized species is slight as shown by its low logP value (- 0.9), indicating low lipophilicity and low passive diffusion; as it exists largely as the hydrophilic cationic species at physiological pH.[2] Lisinopril is reported not to be significantly bound to plasma proteins, this is reflected by its high volume of distribution. It is excreted unchanged in the urine.



Some analytical methods for determination of lisinopril in biological fluids have been reported in the literature. An LC-MS/MS method was developed and validated for pharmacokinetic evaluation of lisinopril in Wister rat's plasma. The data indicate that bioavailability of lisinopril was approximately 5.4 %. They attribute the poor bioavailability to low permeability and thus poor absorption in the experimental animals used.[4] A HPLC method for the determination of lisinopril in dosage forms and spiked human plasma through derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole with fluorimetric detection at 540 nm was reported.[5] The method utilized bumetanide as internal standard (IS) with mobile phase consisting of methanol-0.02M disodium hydrogen phosphate pH 3.0 in ratio 55:45. However, their method was applied for the in vitro determination of the drug in spiked human plasma thus, its application in biological fluids was suggested since the method have a good LOD of 0.008 µg/mL. Another sensitive HPLC method for the determination of lisinopril in human plasma and urine with fluorescence detection at 477 nm was reported.[6] No internal standard to overcome sample-to-sample variation was used in this method which they conclude was unnecessary since good reproducibility was obtained.[6] The retention time (11.5 minutes) of lisinopril reported was rather bit long. However, the limit of detection (LOD) of the method was satisfactory. An LC-MS method that successfully resolved lisinopril from hyoscyamine (IS) in serum with retention times of 4.33 and 5.29 minutes respectively was reported.[7] The mobile phase is ternary consisted of 50 mM ammonium formate buffer (pH 3)-acetonitrile-methanol in ratio (72:7:21). The column is C18 (250 × 3.2 mm id) which was flushed throughout the experiment with acetonitrile-water (60:40) to maintain its efficiency due to utilization of buffer. The method's LOD and limit of quantification (LOQ) was very low reflecting its poor sensitivity which may discount its applications in pharmacokinetic and bioavailability studies in addition to the tedious processes. A HPLC method for simultaneous determination of metformin and three ACE inhibitors (lisinopril, captopril and enalapril) utilizing caffeine as internal standard was reported.[8] The retention times of the analysed drugs were not mentioned and the displayed chromatogram makes it difficult to ascertain the actual retention time as the chromatogram was not duly labelled. To the best of our literature search, there has been no report on validating analytical method for determination of lisinopril in human saliva; a simpler medium to handle compared to blood whose collection does not involve invasive procedure. This research is aimed at developing and validating a RP-HPLC method for the determination of lisinopril in human saliva with the hope that can be employed for pharmacokinetic and bioequivalence studies involving the drug in the future.

MATERIALS AND METHODS

Equipment and reagents

Standard lisinopril powder, caffeine standard powder, HPLC grade methanol and water obtained from Sigma Aldrich (Germany). HPLC column: Chemsl ODS C18 (200 mm×4.6 mm i.d., 5µ particle size), Shimadzu D439300179 digital analytical weighing balance, Thermo Electron Corporation Centra CL2 centrifuge, HPLC sample bottles 1.5 mL, HPLC used was Agilent technologies (Model 1200 Infinity Series). FTIR machine (Agilent technologies model 1260 Infinity Series). A double scanning UV/vis spectrophotometer (Model SP 3000) was also used.

Preparation of solutions

Preparation of suitable dissolution solvent for lisinopril standard powder

Although lisinopril is highly soluble in water, it was observed that the solvent that gives better resolution both for the drug and internal standard (caffeine) is methanol:water (M:W) in a ratio 50:50 v/v. Hence the solvents was used to dissolve the lisinopril and IS for the purpose of the analysis.

Preparation of stock solution of lisinopril

A stock solution (100 µg/mL) of lisinopril was prepared by weighing accurately 2 mg of lisinopril powder and dissolving in 20 mL of M:W.

Preparation of stock solution of the caffeine (IS)

A stock solution (100 µg/mL) of caffeine was prepared by weighing accuratey 2 mg of caffeine powder and dissolving in 20 mL of M:W.

Chromatographic conditions

Chromatographic separation was achieved using Chemsl ODS® C18 (200 mm × 4.6 id). The mobile phase consists of methanol-water containing 0.1 % orthophosphoric acid as additive. Isocratic separation conditions were achieved through varying and optimization of mobile phase ratios, injection volume, temperature, detection wavelength and flow rate after several trials. The optimized conditions that allowed for the detection and resolution of lisinopril and caffeine (IS) with ample sensitivity were adopted.

Preparation of lisinopril-saliva sample

A solution of lisinopril (12.5 µg/mL) was prepared from the stock solution, a portion (2 mL) of which was added to blank saliva (2 mL) followed by 1 mL of a solution (0.05 µg/mL) of the caffeine (IS) to obtain a mixture containing 5.0 µg/mL of lisinopril. The mixture was vortex mixed and centrifuged at 3000 rpm for 10 min which gives a relatively clear solution. A quantity (0.5 mL) of the clear solution was injected into the HPLC operated at the optimized chromatographic conditions.

Preparation of calibration curve of lisinopril

Various solutions (2.5, 6.25, 12.5, 25.0, 50.0, 62.5 and 125.0 µg/mL) were prepared from the stock solution and portions (2 mL each) were transferred into series of 5 mL volumetric flask each containing blank saliva (2 mL) followed by addition of 1 mL of a solution (0.05 µg/mL) of the caffeine (IS) to obtain mixtures containing of 1.0, 2.5, 5.0, 10.0, 20.0, 25.0 and 50.0 µg/mL respectively of lisinopril and 50 µg/mL of the caffeine (IS). These mixtures were vortex mixed and centrifuged at 3000 rpm for 10 min resulting in clear solutions. A quantity (0.5 mL) of each clear solution was injected into the HPLC machine operated at the optimized chromatographic conditions. The peak height ratios of lisinopril/caffeine (IS) in the respective chromatograms obtained were then plotted against the corresponding concentrations.

Validation of RP-HPLC method

The method was validated with respect to precision, accuracy, percentage recovery, limit of detection and limit of quantitation.[9]

Intraday and interday precision

The intraday precision of this method was determined by taking five replicates analysis of a 10.0 µg/mL solution of lisinopril using the method at 1 hr intervals within the same day. While for the interday precision, five replicates analysis of the solution were done daily using the method for three consecutive days. The percentage coefficient of variation (% CV) in both cases was calculated as a measure of precision of the method.

Accuracy and recovery

The accuracy of this method was checked by standard addition method, where 80, 100 and 120 % of a 10 µg/mL solution of lisinopril were added to same and treated with saliva and the IS as described in the methodology to obtain 18, 20 and 22 µg/mL solutions of lisinopril respectively. The mixtures were centrifuged as described under preparation of calibration curve before finally injecting into the HPLC machine. After obtaining the chromatograms, the lisinopril content was determined by subtracting the peak height ratio of lisinopril/caffeine (IS) of the unspiked solution (10 µg/mL) from that found in each of the spiked solutions and interpolating the final concentrations from the calibration curve. Accuracy was expressed as percentage relative error (% Er) and

percentage recovery.

Limit of detection and limit of quantification

The limit of detection (LOD) was determined by studying the calibration curve using samples containing the drug in the range of LOD. The standard deviation of y-intercepts of the regression lines was used as standard deviation. LOD is expressed as:

$$\text{LOD} = \frac{3.3\sigma}{S},$$

The limit of quantitation (LOQ) was determined using the expression:

$$\text{LOQ} = \frac{10\,\sigma}{S}$$

Where σ in each case is the standard deviation of y-intercepts of the regression lines determined through LINEST function in Microsoft Office Excel® 2016

RESULTS

The optimized chromatographic conditions of the developed RP-HPLC method are shown in Table 1 while the chromatograms obtained are presented in Figures 2, 3, and 4. Calibration parameters for RP-HPLC method are shown in Table 2. Calibration curve for lisinopril in saliva is shown in figure 5. The results of the validation parameters for the RP-HPLC method are shown in Table 3.

Table 1: Optimized chromatographic conditions of the method

Parameters	Descriptions
Mobile phase	Methanol: Water (80:20)
Column	Chemsl ODS® C18
Column size	200 mm×4.6 mm i.d., 5µ particle size
Additive	0.1% orthophosphoric acid
Detection wavelength λ	218 nm
Column temperature	35°C
Flow rate	10 µL
Injection volume	1 mL/min
Runtime	4 min
Retention time	1.784 min

Table 2: Calibration curve parameters of the developed method

Parameter	Value obtained
Linearity range (µg/mL)	1.0 – 50.0
Correlation coefficient (r)	0.9981
Regression equation	A = Cy + x
Slope (y)	0.147
Intercept (x)	0.0984

Table 3: Validation parameters of the developed method

Parameter	Value obtained
Precision Intraday (% RSD) ± SD	0.6 ± 0.16
Interday (% RSD) ± SD	0.8 ± 0.12
Accuracy ± SD (% Er)	1.20 ± 0.20
Recovery ± SD (%)	99.89 ± 0.20
Limit of detection (ng/mL)	0.15
Limit of quantification (ng/mL)	0.46

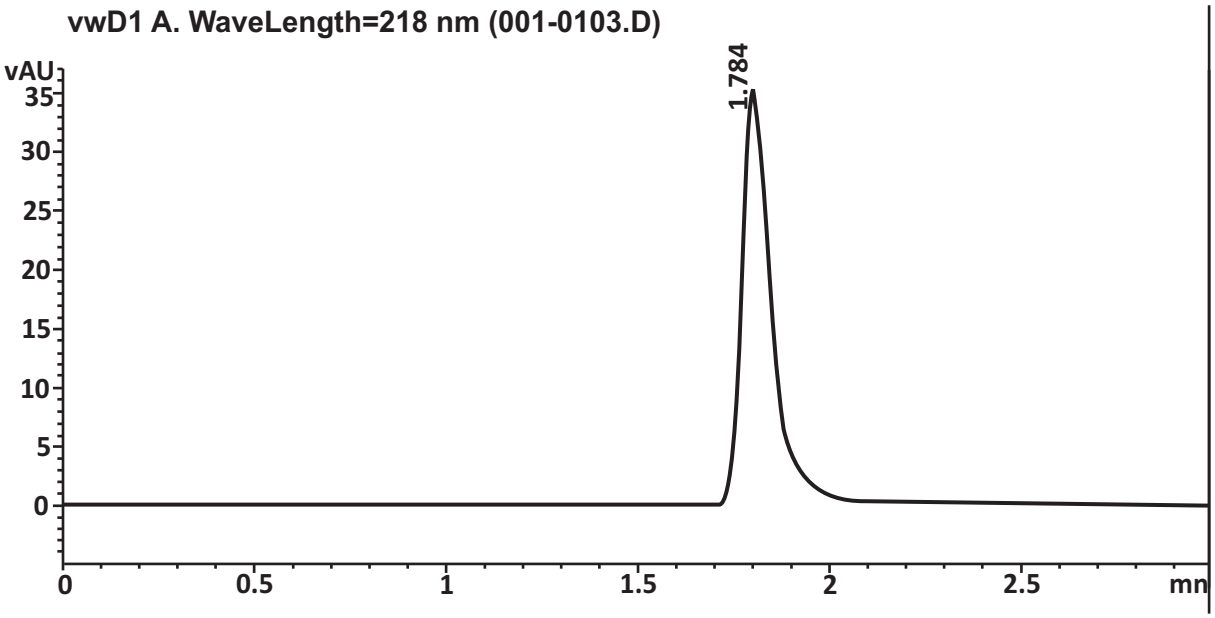


Figure 2: RP-HPLC chromatogram of lisinopril (5 µg/mL)

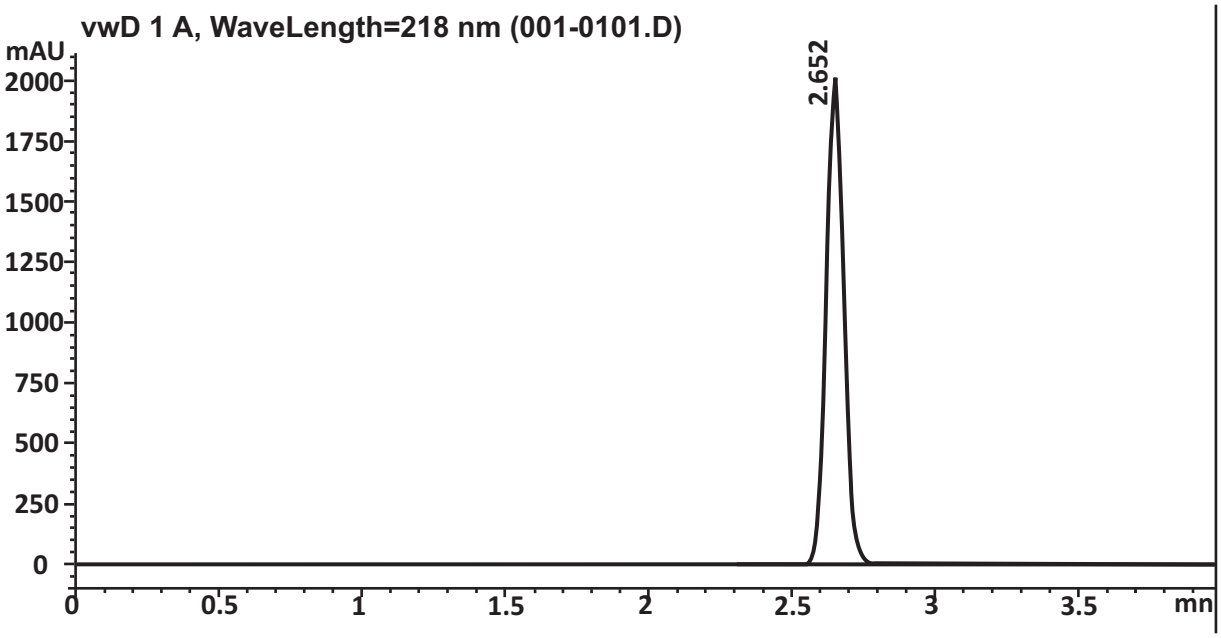


Figure 3: RP-HPLC chromatogram of caffeine (IS) (50 µg/mL)

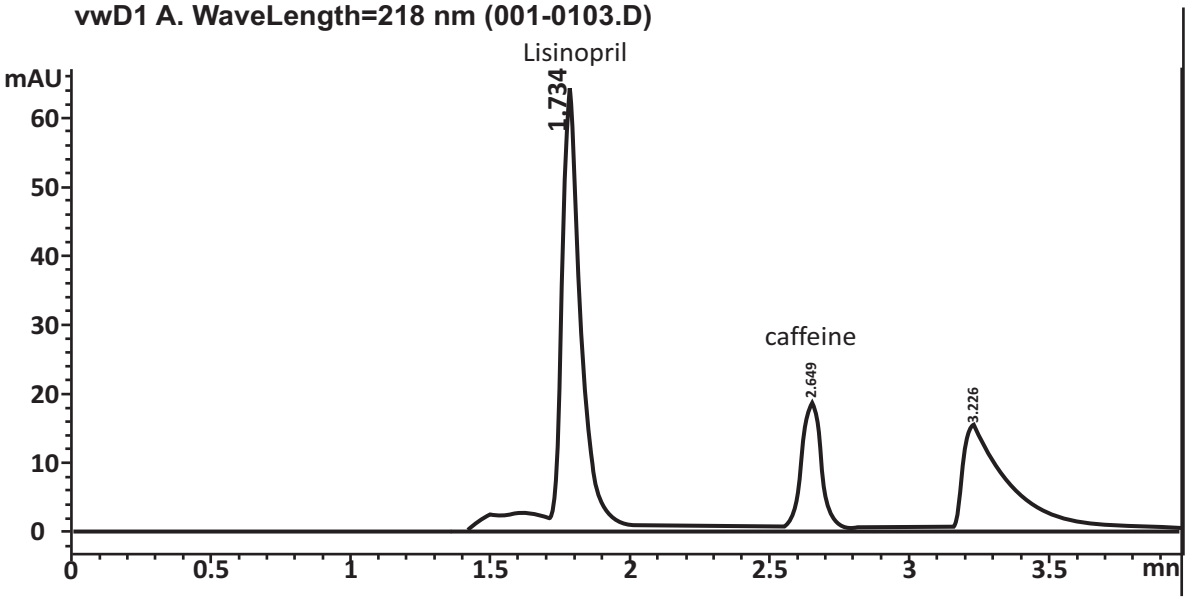


Figure 4: RP-HPLC chromatogram of lisinopril and caffeine (IS) spiked saliva

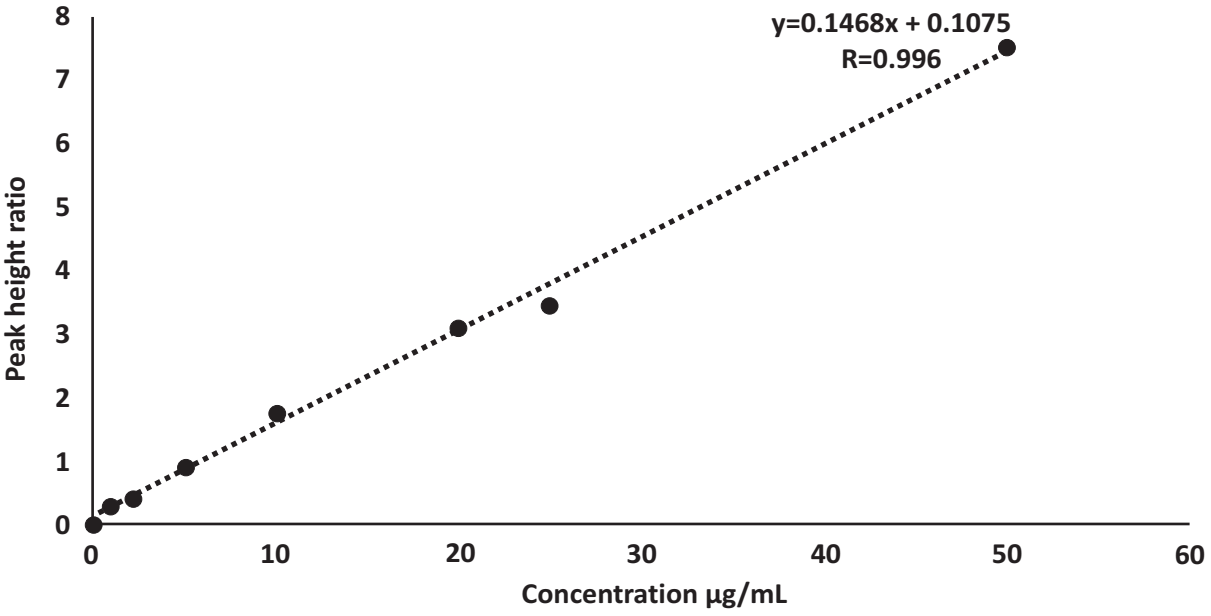


Figure 5: RP-HPLC calibration curve of developed method for lisinopril

DISCUSSION

The linear relationship between the peak height signals (A) and their corresponding concentrations (C in ng/mL) is given by the regression equation of the type A = Cy + x (Table 2). The coefficient of correlation (r) is approaching unity (0.998). This shows a direct proportional relationship between peak height signals and concentrations.

The mobile phase being the polar part of reverse phase HPLC system always has a profound effect on the separation of drug molecules which are mostly polar in nature. Different ratios of methanol-water were used to observe the resolution between lisinopril and caffeine (IS). The best resolution was achieved with a solvent system composing of methanol and water (80:20 v/v); thus was adopted as the mobile phase. Both lisinopril and caffeine (IS) absorbed well at a wavelength of 218 nm when scanned with UV in various compositions of the mobile phase, thus it was selected as optimum wavelength. The peak parameters such as height, asymmetry and tailing were considered while maintaining flow rate, baseline drift etc.

The uniformity of the system operation throughout the analysis was checked by initially equilibrating the column with mobile phase prior to injection of the sample into the chromatographic system. Theoretical plates, tailing factor, resolution and repeatability were checked prior to starting analytical work every time. All the factors were found satisfactory and in accordance with the guidelines.[9]

The low % RSD (≤ 0.80) obtained shows the precision of the method. With good technique and reliable methodology, the precision should be < 15 % CV.10 A % RSD of 3.81 and 4.14 was reported in HPLC method for determination of lisinopril in human plasma and urine,[6] similarly a % CV of ≤ 5.26 was reported.[11] This shows that the precision of the developed method is satisfactory. The accuracy (≤ 1.2) of the method expressed as the measure of percentage relative error are within the range (1 - 5 %) for moderately accurately procedure.[10] The average percentage recovery for the method was found to be 99.89 % showing that the method has good recovery especially when compared with the 78.25 % reported in a HPLC method for estimation of lisinopril,[6] similarly a percentage recovery of 96.6 % - 103.1 % for lisinopril in LC-MS/MS method for the simultaneous determination of lisinopril and hydrochlorothiazide in human plasma was reported.[11] The LOD (≤ 0.15 ng/mL) and LOQ (≤ 0.46 ng/mL) for the developed method are satisfactory. An LOD of 0.028 µg/mL for lisinopril in RP-HPLC method for simultaneous determination of the drug with other drugs in dosage formulation and in human serum.[12] The range of values of the analytical parameters affirms that the developed method was sensitive enough for the analysis of the drug in human saliva.

CONCLUSION

The developed method is simple, accurate and reproducible, and all its validation parameters fall within the acceptable limit for a good analytical method. Hence the method can be applied for quantitative estimation of lisinopril in pharmacokinetic, interaction and bioequivalence studies.

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