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Identification and Quantification of Lisinopril from Pure, Formulated and Urine samples by Micellar Thin Layer Chromatography

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Abstract: A simple, selective and economical micellar thin layer chromatographic method for on-plate analysis of lisinopril from pure, formulated and spiked urine samples was developed. The proposed method involves use of silica gel H layers as stationary phase and 4% aqueous N-cetyl-N, N, N-trimethylammonium bromide (CTAB) as solvent system. The nature as well as the concentration of surfactants influences the mobility of lisinopril. The effects of alkanols usually used as organic modifiers in the solvent system, pH of the solvent system and the presence of nonelectrolytes (organic) and electrolytes (inorganic) in the solvent system on the mobility of lisinopril were studied. The interference study was carried out by using various organic and inorganic metabolites usually present in human urine. The spectrophotometric determination of lisinopril (pure, formulated and spiked urine) samples was carried out at 595nm using ninhydrin as chromogenic reagent. The beers law is obeyed in a concentration range of 10-150 g/mL with correlation coefficient of 0.9778 and molar absorptivity of 4.083×10^3 mol^{-1} cm^{-1}. The recoveries of lisinopril (pure, formulated and urine spiked) were within range of 93.0 -100.2% with relative standard deviation ranging from 0.90 -2.8 %.

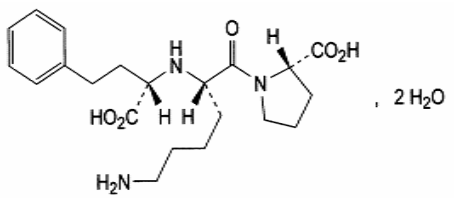
Keywords: Micellar thin layer chromatography, Lisinopril, Urine, Surfactants, Spectrophotometer

Introduction

Lisinopril, 1-[N- {(s)-I- carboxy- 3 phenyl propyl}-L-proline dehydrate (Fig-1) is a lysine analog of enalaprilat, the active metabolite of enalapril. It is long-acting, nonsulhydryl angiotensin- converting enzyme (ACE) inhibitor that is used for the treatment of hypertension and congestive heart failure in daily dosage 10-80 mg [1]. Pharmacological activity of lisinopril has been proved in various experimental and clinical studies [2,3]. Owing to its importance and widespread use, efforts have been made towards the development of simple and reliable analytical methods. As per our literature survey, lisinopril in pharmaceutical formulations has been determined by various analytical methodologies like polaragraphy[4], potentiometry [5] and spectrophotometry [6], but most of these analytical methods are not too suitable for the Identification of lisinopril from clinical samples because of the interferences caused by the amino acids and amino groups containing metabolites present in biological samples [7]. This report is an attempt in the direction of developing a simple and reliable method for on plate identification and quantification of lisinopril in pharmaceutical formulations as well as from human urine samples using silica gel H layers developed with a new mobile phase comprising of micellar solutions of N-cetyl-N, N, N- trimethylammonium bromide (CTAB). Micellar solutions have found numerous practical applications in many areas of separation science. Micellar liquid chromatography (MLC) has gained immense popularity and wider applicability due to operational simplicity, cost effectiveness, relatively non toxicity and enhanced separation efficiency, low aggressiveness [8-11]. Incorporation of aqueous micellar solutions as mobile phase was pioneered by Armstrong and Terrill [12] as they accentuated the importance of TLC where simultaneous separation of ionic or non-ionic species in a variety of matrices is required. A peculiarity of the micellar mobile phases (MMPs) is that they have no macroscopic analogues [13], as a result the typical separations can be easily achieved by using MMPs than aqueous organic mobile phases. Previously MMPs were successfully employed in TLC based critical separations of aromatic hydrocarbons [14], nucleotides [15], vitamin K_1 and K_5 [16], o-,m- and p- aminophenol [17], amino acids [18], separation of penicillins [19].

The human urine analysis for identification of selected drugs and their metabolites has emerged as an important investigation tool in forensic drug analysis [20]. Among all chromatographic methods available only thin layer chromatography (TLC) enables a simple fast and effective separation of the complex mixtures present in various biological samples [21] and is recommended as a approved testing for forensic drug analysis by federal Law [22]. TLC proved its applicability during successful separation of bio-active amines [23], carbohydrates [24], enzymes [25], porphyrins and their precursors [26], alkaloid and drugs [27] from urine samples.

Figure No. 1: Structure of lisinopril drug.



Experimental

Instrumentation and reagents

A UV-VIS Spectrophotometer connected with P IV IBM computer, TLC applicator (Toshiwal India) and pH meter Elico India Ltd was used. Chemical required like silica Gel 'H', sodium dodecyl sulfate (SDS), N-cetyl-N, N, N-trimethylammonium bromide (CTAB) and t- octyl phenoxydacaethoxy ethanol (TX-100), methanol, ethanol, prpoanol and butanol, ninhydrin, isoamyl-alcohol, ethylene dichloride were purchased from Merck India, Iodine crystals, glacial acetic acid , dimethyl formamide (DMF) were

obtained from CDH India, Lisinopril (pure & formulated) was from Lupin labs, Mandideep, M.P., India as a gift sample.

General procedure

Preparation of Test Solution

Ten tablets equivalent to 100 mg of the lisinopril were powdered and transformed into a 50 mL standard flask and dissolved with 50 mL of methanol followed by stirring. The solution was filtered with Wattman no-41 and the filtrate was transferred again into a 100 mL standard flask and diluted to volume with DMF. In case of pure lisinopril drug the 100 mg of powder was dissolved with 10 mL double distilled water and the final volume (100 mL) was made up with DMF. Standard lisinopril solutions (2mg/mL and 1-100µg mL) were prepared from above prepared stock solution by appropriate dilution with DMF and water (9:1).

Preparation of ninhydrin solution

2% solution of ninhydrin was prepared in DMF.

Extraction of Lisinopril from human urine samples

Preparation of spiked drug urine samples –

A sample of Urine was taken from a healthy person. Take 100 mL of this urine sample and adjusted to pH 7-8 with 1 M NaOH solution. Add 100 mg of lisinopril drug powder to the urine sample. The resulting mixture was kept for shaking (20 minutes) at room temperature (15 - 20 ^{0}C) for achieving complete dissolution of drug in urine.

Isolation of drug from spiked drug urine samples

The 100 ml of spiked urine sample was filtered with whatman filter paper (No-41) and then the filtrate was mixed with 400 mL of ethylene dichloride containing 10% of isoamyl alcohol which was kept for shaking for 15 mins. After this the organic layer was separated and evaporated to obtain lisinopril drug as residue. The residue was diluted with 50 mL of DMF: double distilled water mixture (9:1). The chromatography of the extracted drug (lisinopril) was performed on silica gel H layers with solvent system M_5 and the R_F value of spiked lisinopril is compared with R_F value of pure lisinopril drug.

Detector Iodine Vapors were used as a detector.

Stationary Phase Silica Gel 'H' layers were used as a stationary phase.

Mobile phase

The solvent systems were used (Table 1) as mobile phases.

Chromatography

(a) Preparation of TLC plates

The TLC plates were prepared by mixing silica gel H with double distilled water in 1:3 ratio by weight with constant shaking to obtain homogeneous slurry. The resultant slurry was applied on the glass plates with the help of a manual applicator to give a 0.25 mm-thick layer. The plates were dried at room temperature and then activated at 100 ± 2 ^{0}C by heating in an electrically controlled oven for one hr. The activated plates were stored in a close chamber at room temperature until used.

(b) Chromatographic procedure

Test solutions (10µL) were applied on (15 × 3 cm) silica gel H thin layer plates with the help of micropipette at about 2 cm above the lower edge of the plates. The solvent ascent was fixed to 10 cm in all cases for the determination of R_F values of all individual drugs. Linear ascending development was carried out in a vapor equilibrated TLC twin trough chamber. The optimized chamber saturation time for the mobile phase was 15 min at room temperature (25 ± 1 ^{0}C). Subsequent to the development, TLC plates were dried at room temperature. The plates were then detected by using iodine vapors and all the drugs are visualized as colored spots. The R_F values of drug were determined by the following relation –

$R_F = 0.5 (R_L + R_T)$

where $R_L = R_F$ of leading front.

$R_T = R_F$ of trailing front.

Interference

For investigating the interference of various metabolites like sodium and potassium salts, urea and liquor ammonia, normally found in human urine on mobility of lisinopril. An aliquot (5 L) of lisinopril was spotted on silica gel H TLC plate followed by spotting of 5µL of the interfering species (1mg/mL) on the same spot. The chromatography was performed with solvent system M_5. The spots were detected and the R_F values of drug were calculated and compared.

Quantitative determination of lisinopril

The visible spectrophotometeric technique was applied for the quantitative determination of on plate identified lisinopril drug from formulated and human urine spiked samples. For this purpose 10µL of lisinopril solutions of different strength (10 -30µg) were spotted on TLC plates. After complete drying of the spots, the TLC plates were developed with solvent system M_5. At the same time, a pilot plate was also developed to locate the position of lisinopril spot. After such development the area was scarped from the plate. The scraped silica powder was then mixed with 2 mL of DMF for the extraction of lisinopril from adsorbent. Then it was filtered with whatman filter paper no-41. In order to ensure complete extraction of drug the adsorbent was again washed with 3 mL of DMF: double distilled water mixture (9:1). All the filtrate was collected in a test tube, and then 1.5 mL of ninhydrin solution was added to it and kept for 90 min at room temperature for complete color development. The absorbance was measured at 595 nm against reagent blank[22]. A calibration curve was plotted between the absorbance Vs concentration of lisinopril drug. The content of lisinopril in the formulated and urine spiked samples was determined from the standard curve by six replicate readings under similar conditions by using the following relationship:

$$\text{Relative Recovery (\%)} = 100 - \frac{\text{Amount recovered} - \text{Amount loaded}}{\text{Amount loaded}} \times 100$$

Results and discussion

The mobility of lisinopril drug (pure and formulated) was examined on silica gel H layers using aqueous solutions of cationic (CTAB), anionic (SDS) and nonionic (TX-100) surfactants. The experimental conditions were optimized on the mobility of lisinopril with various factors, such as type and concentration of surfactants used, nature and concentration of added alkanols in the micellar mobile phases, acidity or basicity of the medium, presence of urea and NaCl (electrolyte) in the surfactant- containing mobile phase and effect of various organic and inorganic metabolites normally found in urine samples.

Effect of type and concentration of surfactants

The chromatography of lisinopril was performed on silica gel H layers using different concentrations of cationic, anionic and nonionic surfactant-mediated mobile phase systems (M_2- M_{19}). The results of the effect of type and concentration of different classes of surfactants are presented in Table 2. The following conclusions are drawn from the Table 2.

(1) In double distilled water (zero concentration of surfactant), the lisinopril remains at the point of application.

(2) The mobility of lisinopril increases with the increase in concentration of CTAB in the solvent systems.

(3) Reverse trend was observed in case of SDS as compared to CTAB, the mobility of lisinopril decreases with the increase in concentration of SDS in solvent system.

(4) The same trend was obtained in case of Triton X-100 as observed in case of CTAB containing solvent system. The mobility of lisinopril increases with the increase in concentration of Triton X-100 in the solvent system.

It may be concluded from the present study that in case of both CTAB and Triton X-100, the micellar thin layer chromatography is involved. The surfactant in the solvent system occurs in both the micellar and ionic forms. In this case concentration of surfactants in the mobile phase leads to an increase only in the concentration of micelles in MMPs and the concentration in the stationary phase remains nearly constant. This may result in decrease in retention of adsorbates. While in case of SDS, the ion-pair TLC situation is observed. The mobile phase in the system contains only ions of a surfactant. An increase in their concentration in the mobile phase increases the concentration of surfactant ions adsorbed on the stationary phase. As a result the retention of adsorbates increases [28].

Effect of alkanols

The different concentrations of alcohols (methanol, ethanol, n-propanol, or n-butanol) of varying chain lengths were used with the 4% aqueous CTAB (M_5) for the chromatography of lisinopril on silica gel H layer. The effect of nature of alcohols is presented in Table-2. From the results obtained, it is clear that with the increase in the concentration of alcohols the mobility decreases. At higher concentrations of different alcohols the visibility of lisinopril decreases and is not detected. The addition of alcohols in the micellar solvent systems may result in the less population of surfactants molecules on the adsorbent and this may provide some free silanol groups on the silica surface for the adsorption of lisinopril [29]. This may cause the increase in the retention of lisinopril on the silica surface and hence decreases the mobility.

Table1: Solvent systems used.

Code	Constituents
M_1	Water
M_2	0.5% aqueous CTAB
M_3	1% aqueous CTAB
M_4	2% aqueous CTAB
M_5	4% aqueous CTAB
M_6	5% aqueous CTAB
M_7	7% aqueous CTAB
M_8	0.5% aqueous SDS
M_9	1% aqueous SDS
M_{10}	2% aqueous SDS
M_{11}	4% aqueous SDS
M_{12}	5 % aqueous SDS
M_{13}	7% aqueous SDS
M_{14}	0.5% aqueous TX-100
M_{15}	1% aqueous TX-100
M_{16}	2% aqueous TX-100
M_{17}	4% aqueous TX-100
M_{18}	5 % aqueous TX-100
M_{19}	7% aqueous TX-100
M_{20}	4% aqueous CTAB + Methanol (9 : 1)
M_{21}	4% aqueous CTAB + Methanol (8 : 2)
M_{22}	4% aqueous CTAB + Methanol (7 : 3)
M_{23}	4% aqueous CTAB + Methanol (6 : 4)
M_{24}	4% aqueous CTAB + Methanol (5 : 5)
M_{25}	4% aqueous CTAB + Methanol (4 : 6)
M_{26}	4% aqueous CTAB + Ethanol (8 : 2)
M_{27}	4% aqueous CTAB + Ethanol (5 : 5)
M_{28}	4% aqueous CTAB + Ethanol (2 : 8)
M_{29}	4% aqueous CTAB + Popanol (8 : 2)
M_{30}	4% aqueous CTAB + Popanol (5 : 5)
M_{31}	4% aqueous CTAB + Popanol (2 : 8)
M_{32}	4% aqueous CTAB + Butanol (8 : 2)
M_{33}	4% aqueous CTAB + Butanol (5 : 5)
M_{34}	4% aqueous CTAB + Butanol (2 : 8)
M_{35}	4 % aqueous CTAB containing 1 g NaCl per 100 mL
M_{36}	4 % aqueous CTAB containing 5 g NaCl per 100 mL
M_{37}	4 % aqueous CTAB containing 1 g Urea per 100 mL
M_{38}	4 % aqueous CTAB containing 5 g Urea per 100 mL
M_{39}	4% aqueous CTAB in buffer solution of pH 2.3
M_{40}	4% aqueous CTAB in buffer solution of pH 4.2
M_{41}	4% aqueous CTAB in buffer solution of pH 5.7
M_{42}	4% aqueous CTAB in buffer solution of pH 9.1

Effect of acidity and basicity of the solvent systems used

TLC of lisinopril was performed with solvent systems M_{39}- M_{42} prepared by using 4% aqueous CTAB in borate phosphate buffers of different pH values (pH -2.3, 4.2, 5.7 and 9.0). The results summarized in table- 2, shows that at strongly acidic pH no spot was detected for lisinopril, while at pH (4.2 and 5.7) a decrease in the mobility of drug was observed with compact spots. At pH 9.0 a slight increase in mobility was observed for lisinopril drug with an elongated spot.

Effect of electrolytes and non electrolytes addition in solvent systems

It has been reported [30] that the microenvironment of micellar system is greatly influenced by the presence of added organic substance or inorganic electrolytes. Thus the effect of addition of urea (organic non electrolyte) andNaCl

(inorganic electrolyte) at two different concentration levels in the solvent system M_5 (4% CTAB) on mobility of lisinopril were examined. The results presented in Table-2 and from the available data following trends are noticeable –

(1) A tailed spot was observed at lower concentration of urea but at higher concentration no spot was detected.

(2) In case of NaCl mobility of lisinopril increases but at higher concentration spot compactness and intensity decreases.

Table 2: R_F values (mobility) of lisinopril on silica gel H layers with different (M_1-M_{42}) mobile phases.

Mobile Phases	R_F Values	
	Lisinopril (Pure)	Lisinopril (formulated)
M_1	0.00	0.02
M_2	0.20	0.18
M_3	0.24	0.22
M_4	0.27	0.29
M_5	0.32	0.35
M_6	0.35	0.36
M_7	0.36	0.38
M_8	0.28	0.29
M_9	0.26	0.25
M_{10}	0. 24	0.23
M_{11}	0.21	0.21
M_{12}	0.20	0.19
M_{13}	0.20	0.18
M_{14}	0.24	0.22
M_{15}	0.26	0.25
M_{16}	0.29	0.30
M_{17}	0.33	0.35
M_{18}	0.34	0.35
M_{19}	0.34	0.34
M_{20}	0.46	0.44
M_{21}	0.42	0.41
M_{22}	0.39	0.37
M_{23}	0.36	0.34
M_{24}	0.31	0.30
M_{25}	n..d.	n.d
M_{26}	0.49	0.51
M_{27}	0.44	0.43
M_{28}	n.d	n.d
M_{29}	0.50	0.53
M_{30}	0.48	0.49
M_{31}	n.d	n.d
M_{32}	0.49	0.50
M_{33}	0.46	0.48
M_{34}	n.d	n.d.
M_{35}	0.36	0.35
M_{36}	0.38	0.38
M_{37}	0.37 (T)	0.35 (T)
M_{38}	n.d.	n.d.
M_{39}	n.d	n.d.
M_{40}	0.31	0.28
M_{41}	0.29	0.25
M_{42}	0.36	0.36

(T)- Tailed spot

Identification of lisinopril in presence of impurities

To widen the applicability of the developed TLC system (silica gel H layers with M_5 solvent system) for identification and isolation of lisinopril from formulated drug and urine samples in presence of various metabolites normally found in urine are presented in Table-3. It is clear that potassium and sodium salts does not affect the mobility of the drug, but in case of liquor ammonia a long trailing spot was observed . In case of urea two spots were observed.

Table 3: Effect of various organic and inorganic metabolites found in urine on mobility (R_F) of lisinopril Drug.

Impurity	R_F Value
NaCl	0.34
KCl	0.35
Urea	0.33, 0.68
Liquor Ammonia	0.39 (T)

(T) Trailing

Table 4: Optical characteristics and statistical data for the regression equation of the proposed method

Sr. No	Parameter	Value
1	λ_max	595 nm
2	Beer's law limit (µg/mL)	10-150
3	Molar absorptivity (L mole-1 cm-1)	4.083×10^3
4	Sandell's sensitivity (µg/mL per0.001 A)	1.2×10^{-1}
5	Regression equation (Y*)	
6	Slope (m)	6.28×10^{-3}
7	Intercept (c)	2.8×10^{-3}
8	Correlation coefficient (r^2	0.9778
9	Relative Standard Deviation** (%)	0.883
10	Limit of Detection (µg/mL)***	5.587

$Y^* = mx + C$

Where X is the concentration of analyte (µg/mL) and Y is absorbance unit.

** = Calculated from six determinations

*** Calculated as per ICH guidelines

Identification of lisinopril from human urine samples

The proposed chromatographic method (silica gel H layers with 4% aqueous CTAB) successfully identified the lisinopril extracted from the human urine samples on the basis of R_F value. The spots of the pure, formulated and the urine extracted lisinopril samples are shown in Fig 2. Thus the proposed method is very sensitive for the identification of lisinopril from urine samples.

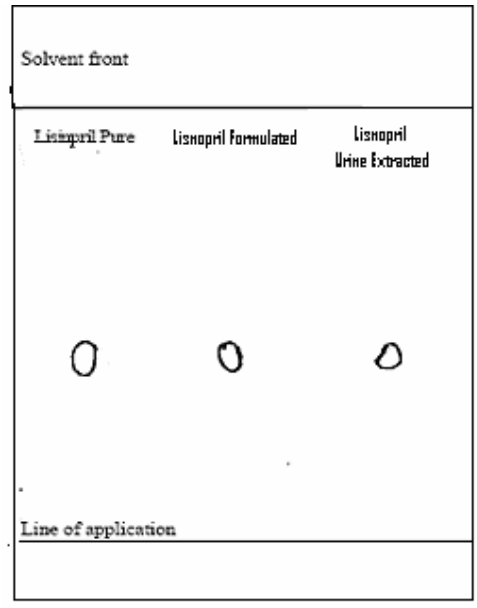
TLC-Spectrophotometry of lisinopril

The analytical parameters for the spectrophotometric determination of lisinopril by the proposed method are given in table-4. Linear correlation was found between the absorbance and the concentration of lisinopril. Beers law was obeyed in a concentration range from 10-150 µgm/mL. The regression analysis of beer law data using the method of least square was made to evaluate the slope (b), the intercept (a), correlation coefficient (r^2), molar absorptivity and sandell sensitivity for each system. These values suggested that the proposed method is very sensitive for the determination of lisinopril. The accuracy and validity of the proposed method were ascertained by performing recovery studies. The recovery studies of pure, formulated and urine spiked lisinopril samples at different concentrations indicates that the recovery was good. The percentage recovery values ranged between 93.0 -100.2% with relative standard deviation of less than 3%.

Table 5: Spectrophotometric determination of lisinopril from pure formulated and urine spiked samples.

Drug samples (µg/ml) Amount taken	Found (µg/ml)	Relative Recovery (%)	Relative Error (%)	R.S.D
10	9.3	93.0	-6.9	2.8
12	11.2	93.3	-6.6	2.0
14	13.2	94.2	-5.7	2.2
16	15.5	96.8	-3.1	2.6
18	17.3	96.1	-3.8	1.9
20	19.4	97.0	-3.0	1.7
22	21.6	98.1	-1.8	2.9
24	23.7	98.7	-1.2	1.5
26	25.6	98.6	-1.15	1.6
Formulated drug				
50	50.3	100.6	+0.59	1.3
100	100.2	100.2	+0.20	0.9
Urine sample				
20	18.8	94.0	-5.9	2.3

Figure No. 2: Chromatogram showing identification of lisinopril in pure and formulated as well as spiked urine samples on silica gel H layers developed with mobile phase M_5.



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