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RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LISINOPRIL AND DIURETICS IN BULK, DOSAGE FORMS AND HUMAN SERUM

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ABSTRACT

A new, simple, specific and precise assay method has been developed and validated on an isocratic RP-HPLC for the simultaneous determination of lisinopril (LIS) and diuretics (hydrochlorothiazide (HCT) and furosemide (FUR)) in bulk, dosage formulations and human serum at 225nm. Chromatographic separation was achieved by using Purospher Star C_{18} (250 mm x 4.6 mm, 5 µm) column, mobile phase consisting of methanol: water: acetonitrile (80:15:5 v/v/v) adjusted to pH 3.0 via phosphoric acid (85%) having a flow rate of 1.0 mL min^{-1} at room temperature. The retention times for lisinopril, hydrochlorothiazide and furosemide were 2.6, 3.2 and 4.1 min, respectively. Calibration curves were linear over range of 0.25-10 µgmL^{-1} with a correlation coefficient ±0.999. LOD and LOQ were in the ranges of 0.68-2.07 µgmL^{-1}. Intra and inter-run precision and accuracy results were 98.32 to 100.7 %. Therefore, the developed method could be used for routine estimation of lisinopril and diuretics (HCT and FUR) alone or in combination in bulk materials, pharmaceutical dosage formulations, human serum and also for the clinical investigations.

Keywords: Lisinopril, diuretics, RP-HPLC, human serum

INTRODUCTION

Lisinopril (Fig. 1), (S)-1-[*N*2- (1-Carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate, is lysine analog of nonsulfhydryl angiotensin converting enzyme (ACE) inhibitor enalaprilat. It is used in the treatment of hypertension and heart failure. It produces its effect by suppression of the renin-angiotensin-aldosterone system, which in turn decrease sodium and water retention. Lisinopril undergoes glomerular filtration, tubular secretion and reabsorption accumulation occurs in patients with renal dysfunction [1].

The combination of angiotensin converting enzyme inhibitors and diuretics is used to treat hypertension. Diuretics are used in hypertension, diabetes insipidus, renal calculi, edema, hypercalcemia, acute and chronic renal failure, and nephrotic syndrome. It reduces the volume of extracellular fluid, enhance the urinary excretion of sodium chloride, and secondarily, increase the volume of urine excreted by kidney. Commonly used diuretics, which are co administered or used in combination with ACE inhibitors, are hydrochlorothiazide (HCT) and furosemide (FUR), which are thiazide and loop diuretics, respectively (Fig 2). Hydrochlorothiazide is a benzothiadiazine widely use in antihypertensive pharmaceutical preparations which decreases active sodium reabsorption and reduces peripheral vascular resistance and furosemide, is a loop diuretic which acts by inhibiting the Na-K-2Cl symporter in the thick ascending loop of henle [2]. Along with their useful effects these combinations have some unwanted effects. Diuretics cause increase steady state levels of angiotensin converting enzyme inhibitors by altering pharmacokinetics of drugs [3, 4].

Survey reveals that diuretic can enhance the effects of angiotensin-converting enzyme inhibitor in antihypertensive therapy as they induce sodium restriction [5-7]. Toussaint *et al,* [8] showed drug interference between captopril and furosemide, which is independent of ACE-inhibition and probably only due to interference in proximal-tubular secretion of both drugs. Between captopril and hydrochlorothiazide no such interactions were observed. But the work done by Bart W. *et al,* showed that by adding hydrochlorothiazide to ACE inhibitor can alter its pharmacokinetics, resulting in

reduced renal function and increased circulating drug levels, which may provide an explanation for the adverse renal effects [9].

Literature study also revealed that various spectrometric and RP-HPLC methods were reported for the individual determination of LIS [10-18], HCT [19-20] and FUR [21-23]. For the simultaneous estimation of LIS and HCT spectrometric and RP-HPLC methods were also reported [11' 24-26]. However no RP-HPLC method has so far reported for the simultaneous estimation of LIS, HCT and FUR. Therefore, in the light of these evidences, we have developed and validated a simple and accurate RP-HPLC method for the simultaneous determination of lisinopril, hydrochlorothiazide and furosemide for the first time. By using the present method we can quantitate the lisinopril alone or in presence of diuretics (HCT and FUR) in bulk materials, pharmaceutical dosage formulations and human serum. This method would also allow more efficient generation of clinical data and can compare the effect of different diuretic with same antihypertensive drugs.

EXPERIMENT

Materials and reagents

All chemicals and reagents were of analytical grade. Lisinopril (purity 99.82%) was a gift from Brooks Pharmaceuticals Labs (Pvt) Limited, Pakistan. Hydrochlorothiazide (purity 99.94%) and furosemide (purity 99.79%) were gifts from Zafa Pharmaceutical Laboratories (Pvt) Ltd and Sanofi Aventis (Pvt) Limited, Pakistan. HPLC grade acetonitrile, methanol and phosphoric acid were obtained from Tedia (USA) and Merck Darmstadt, Germany.

Pharmaceutical dosage form

Lace. (Lisinopril 10 mg tablets by Brooks Pharmaceuticals (Pvt) Ltd), Diuza. (Hydrochlorothiazide 25 mg tablets by Zafa Pharmaceutical Laboratories (Pvt) Ltd**),** and Lasix. (40 mg tablets from Sanofi Aventis Pakistan Limited), were purchased from the local pharmacies. All these drugs had an expiry of not less than one year at the time of study.

Instrumentation

An isocratic high-pressure liquid chromatograph consisted of an LC-10 AT VP Shimadzu pump, a Purospher Start C_{18} column (250 mm x 4.6 mm, 5 µm) was used for separation, and detection was accomplished with variable

wavelength UV/Vis detector SPD - 10AVP system at 225 nm. The mobile phase comprising of mixture of methanol: water: acetonitrile (80:15:5) adjusted to pH 3.0 with phosphoric acid (85 %). The mobile phase was degassed by using DGU-14 AM on-line ultrasonicator, and was filtered through a 0.45 micron membrane filter and was delivered at a flow rate of 1.0 mL min^{-1} The injection volume was 20 µ L. The chromatographic system was integrated using a CBM-102 communication Bus Module Shimadzu with a Pentium. IV PC loaded with Class GC software for data attainment.

Preparation of standard and sample solutions

HPLC method

Stock standard solutions 100 ppm of LIS, HCT and FUR were prepared in 100mL mobile phase as solvent. Working solutions were prepared separately by making serial dilutions from the standard solution to obtain concentration between 0.5-10, 0.125-2.5 and 0.025-0.5 µg mL^{-1} for LIS, HCT and FUR, respectively. These solutions were stored at 20ºC. Once prepared, analyzed daily for inter and intra-day variations of the method. 20 µ L of these solutions were injected into LC system and chromatographed.

Procedure for tablets

Twenty tablets of each formulation were powdered finely and an amount equivalent to 10 mg of LIS, HCT and FUR was weighed and then dissolved in the mobile phase. Solutions were then filtered through ordinary filter paper. The desired concentrations 0.5-10, 0.125-2.5 and 0.025- 0.5 µg mL^{-1} for LIS, HCT and FUR, respectively were obtained by accurate dilution, solutions were then sonicated. Finally, all the solutions were filtered through 0.45 microns membrane filter, in order to separate out the insoluble excepients before chromatographed.

Procedure for human serum

Plasma samples, obtained from healthy volunteers, were collected and stored. To 1.0 ml of plasma, 9.0 ml of acetonitrile was added; the mixture was vortexed for one minute and than centrifuged for 10 minutes at 10,000 rpm and the supernatant was filtered by 0.45-micron membrane filter. An aliquot of serum sample was fortified with LIS, HCT and FUR to achieve final concentration.

RESULT AND DISCUSSION

Development and optimization of isocratic HPLC conditions

The present study was aimed to develop a simple, precise and accurate HPLC method for simultaneous of estimation LIS, HCT and FUR in bulk, dosage form and in serum by using RP-HPLC C_{18} column (Purospher Star). UV detection was carried out at 225 nm as lisinopril and diuretics showed good absorbance at this wavelength. Initially a variety of solvents and solvent mixtures were investigated for the mobile phases finally the mobile phase consisting of methanol, water and acetonitrile in the ratio of (80:15:5, v/v/v) adjusted to pH 3.0 was found to have sharp, well-defined peaks with best separation and resolution having reasonable short run time of 5 min. Peaks were identified using retention times compared with those of standards. The method was validated according to ICH [27] and USP [28]. The validation parameters tested were specificity, accuracy, linearity, precision, and robustness. Better resolution of peaks, sensitivity of the assay, economical and readily available mobile phase, and shorter time required for analysis. All these factors make this method suitable for estimation of lisinopril and diuretics alone and simultaneously in bulk, pharmaceutical dosage forms without any interference. This method also allows us for the efficient generation of clinical data and by this we can compare the effect of different diuretic with same antihypertensive drugs.

System suitability

System suitability is to check and ensure the on-going performance of a chromatographic system (including instrument, reagents, columns, and analysts) for the intended application. The system was equilibrated with the initial mobile phase composition, followed by 10 injections of the same standard. On each day of method validation 10 consecutive injections were used to evaluate the system suitability. The summary of system suitability parameters of the present study is shown in table 1.

Linearity

To confirm whether the system is responding properly for the change in concentration, we have to plot concentration verses area as it gives regression line or coefficient of correlation. For this, linearity was tested with known concentrations of LIS, HCT

and FUR i.e. 0.5, 1.00, 2.50, 5.00 and10.00; 0.125, 0.250, 0.625, 1.250 and 2.500 and 0.025, 0.050, 0.125, 0.250 and 0.5 µg mL $^{-1}$, respectively. For every concentration five runs were performed. Injected concentrations versus area were plotted and the correlation coefficients were calculated which are shown in table 2.

Regression equation and coefficient of correlation for LIS and HCT and FUR were found to be: y = 2629x + 1732, r = 0.9994, y = 4096+4419, r = 0.9993, and y = 4270 + 4197, r = 0.9996 respectively.

Accuracy

Accuracy is how close a calculated value to the real value and it depends on the instrument. Accuracy was determined at three concentrations (4.0, 5.0 and 6.0 ppm) and the percentage standard deviation was determined by calculating peak area. The calculated RSD value is less then 2 for all tested solutions results are summarized in the table 3. The limit for mean recovery is 98-102%, and thus, the method is accurate in nature.

Intraday and inter-day precision

Intra-day precision and inter-day precision were determined by analyzing the concentrations at five levels, which are 0.5, 1.00, 2.50, 5.00 and 10.00; 0.125, 0.250, 0.625, 1.250 and 2.500 and 0.025, 0.050, 0.125, 0.250 and 0.50-µg mL^{-1} for LIS, HCT and FUR respectively. Each concentration was analyzed in triplicate and intra-assay precision was found to be less than 2 % relative standard deviation (RSD) for all samples on all days. Inter-day precision % RSD for analyses conducted after one day was found to be 0.81, 0.08, and 0.074% RSD

Limit of detection and quantitation

Limit of Detection (LOD), Limit of quantification (LOQ) based on standard deviation of the response and the slopes obtained were calculated from the known concentrations of LIS, HCT and FUR. Given in table 6.

Specificity and selectivity

Specificity or selectivity of a method determine that analytes (LIS, HCT, FUR) can separate under given conditions without interferences from other components in the medium as shown in fig. 3. Specificity was also determined by screening four different samples of controlled human serum, which were free from interfering endogenous plasma components. Solutions of placebo, lisinopril and diuretics were checked for interference from common excipients.

Ruggedness

The ruggedness was established by determining LIS, HCT and FUR in dosage formulation and in human serum using same and different chromatographic system and same column by different analysts on different days. The assay results indicated that the method was capable with high precision (table 4).

Robustness

Robustness of the method was accomplished by designed modifications made to the method parameters such as composition, flow rate, pH of the mobile phase, detection wavelength, injection volume and column temperature (table 5) and it was found that the %R.S.D values did not exceed more than 2 %.

Table 1: System suitability parameters

Drugs	Retention time(t_R)	Capacity factor (K')	Tailing factor (T)	Resolution (Rs)	Theoretical plates (N)	Separation factor (α)
LIS	2.6	0	0	0	2075	0
HCT	3.2	0.22	1.99	2.09	1955	0
FUR	4.1	0.57	0	2.78	2035	2.57

LIS: Lisinopril; HCT: Hydrochlorothiazide; FUR: Furosemide

Table 2: Regression characteristics of the proposed method for lisinopril, hydrochlorothiazide and furosemide

Drugs	LIS	HCT	FUR
Conc. range (µg mL^{-1})	0.5-10	0.125-2.5	0.025-0.5
Correlation coefficient (r^2)	0.9994	0.9993	0.9996
Standard error of estimate	1.02	1.15	0.85
Standard error	0.58	0.63	0.47
Intercept	-0.64	1.10	0.92
Slope	2630	4097	4270

LIS: Lisinopril; HCT: Hydrochlorothiazide; FUR: Furosemide

Table 3: Accuracy of the proposed method for lisinopril, hydrochlorothiazide and furosemide

Parameters	Conc. (µgmL^{-1}) Spiked	Assay (spiking method)			Assay in serum		
		LIS	HCT	FUR	LIS	HCT	FUR
Conc. found	4.0	3.95	3.97	3.96	4.01	3.99	3.96
	5.0	4.98	5.03	4.92	4.95	5.03	4.97
	6.0	5.96	5.99	5.98	5.95	5.90	5.96
% Recovery	4.0	98.89	99.25	98.79	98.99	99.75	100.1
	5.0	99.41	100.12	98.32	99.92	98.98	98.69
	6.0	99.33	99.99	99.74	99.88	99.09	100.4

LIS:Lisinopril; HCT: Hydrochlorothiazide; FUR: Furosemide

Table 4: Inter and intra day Precision of the proposed method for lisinopril, hydrochlorothiazide and furosemide (n=6)

Drug	Conc. (μgmL^{-1})	Formulation (%RSD)		Serum (%RSD)
		Intra-day	Inter-day	Intra-day
		variation		
LIS	0.50	0.08	0.033	0.25
	1.0	0.09	0.085	0.085
	2.50	0.02	0.058	0.063
	5.0	0.019	0.052	0.059
	10.0	0.025	0.028	0.061
HCT	0.125	0.091	0.035	0.069
	0.25	0.029	0.028	0.094
	0.625	0.034	0.063	0.086
	1.25	0.036	0.025	0.096
	2.50	0.069	0.061	0.051
FUR	0.025	0.085	0.095	0.063
	0.05	0.03	0.052	0.036
	0.125	0.06	0.080	0.032
	0.25	0.096	0.082	0.081
	0.50	0.076	0.025	0.062

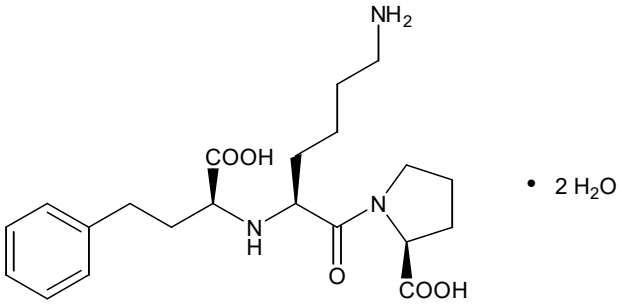
LIS: Lisinopril; HCT: Hydrochlorothiazide; FUR: Furosemide

Table 5: Robustness of the proposed method				
Factors	Level	t_R	K	T
pH				
2.8	-2	2.4	2.45	1.32
3	0	2.6	2.4	1.34
3.2	2	2.8	2.35	1.36
Flow rate (mL min^{-1})				
0.8	-2	2.9	2.35	1.36
1	0	2.6	2.4	1.34
1.2	2	2.4	2.45	1.32
Percentage of methanol in mobile phase (v/v)				
75	-5	3.0	2.3	1.36
80	0	2.6	2.4	1.34
85	5	2.3	2.6	1.32

Table 6 LOD and LOQ of the proposed method for lisinopril, hydrochlorothiazide and furosemide

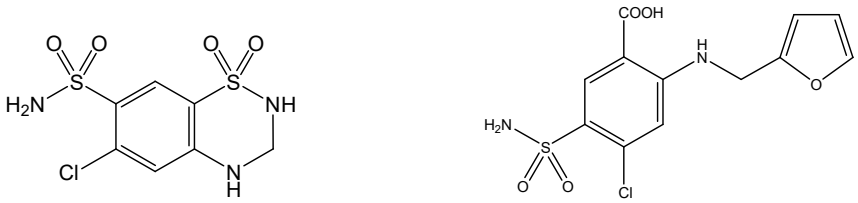
Drugs	Conc. (μgmL^{-1})	LOD (μgmL^{-1})	LOQ (μgmL^{-1})
LIS	0.50-10	0.6842	2.075
HCT	0.125-2.5	0.0378	0.119
FUR	0.025-0.5	0.0493	0.149

LIS:Lisinopril; HCT: Hydrochlorothiazide; FUR: Furosemide; LOD: Limit of Detection; LOQ: Limit of Quantification



Lisinopril

Figure 1: Chemical structure of lisinopril



Hydrochlorothiazid **Furosemide**

Figure 2: Chemical structures Hydrochlorothiazide and Furosemide

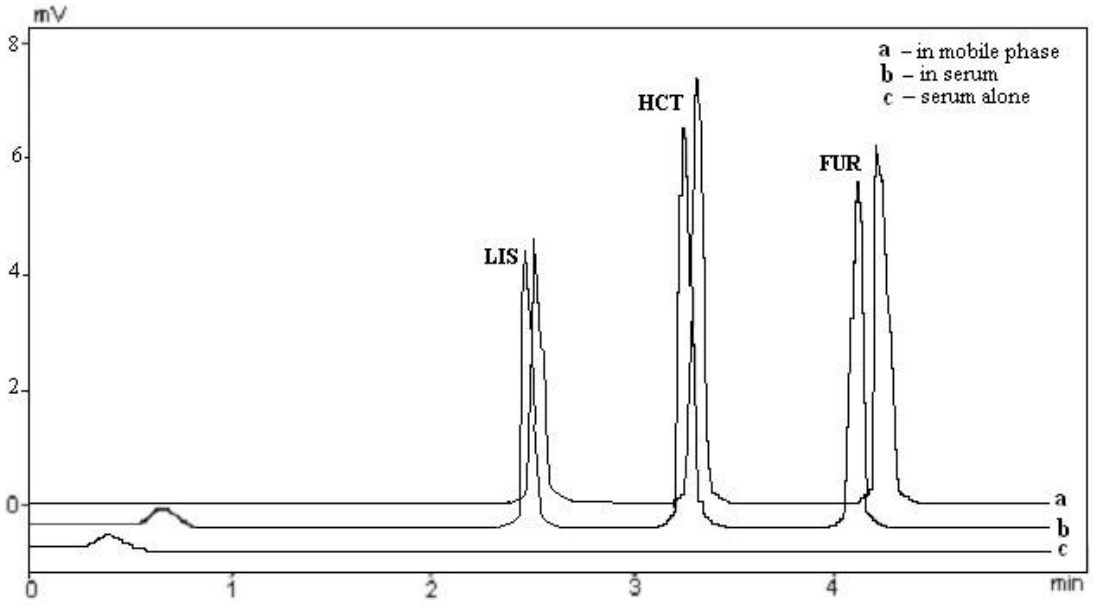


Figure 3: Chromatograms of LIS (50ppm), HCT (12.5ppm) and FUR (25ppm) in mobile phase, human serum, human serum alone and placebo at 225 nm.

CONCLUSION

A simple and reliable HPLC method for monitoring LIS, HCT and FUR in human serum and pharmaceutical dosage formulation has been developed. A fully validated RP-HPLC procedure for the assay of these drugs in bulk, tablets and human serum is described for the first time. Hence, it can be recommended for the routine quality control of these drugs, low volume of blood or plasma is needed. Simplicity of the separation procedure; shorter run time and the low volume of injection make this method suitable for quick and routine analysis. The intra-run and inter-run variability and accuracy results were also in acceptable limit. In addition, this method has the potential application to clinical research of drug combination, multi-drug pharmacokinetics and interactions.

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