RESEARCH ARTICLE

Developed and Validated RP-HPLC Method for Concurrent Analysis of Some Cardiovascular Drugs

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Received: 12th December, 2022; Revised: 19th March, 2023; Accepted: 01st April, 2023; Available Online: 25th June, 2023

ABSTRACT

Objective: An ICH-compliant RP-HPLC approach was created and validated in order to measure the concentrations of HCTZ and LIS in bulk and mixed medicinal dosage forms. This procedure was subsequently submitted for certification.

Methods: Column, a Phenomenex Luna C18(2) (250 x 4.6 mm, 5µ) with a Methanol: Formic acid (30:70) mobile phase, a flow rate is kept as 01 mL/min, the wavelength of detection was 215 nm, and a used detector was PDA.

Results: Hydrochlorothiazide (HCTZ)) and lisinopril (LIS) both had linear calibration curves (r2 = 0.9973 and 0.9983, respectively) for the ranges of concentration 4.0 to 6.0 and 10.0 to 15.0 µg/mL. The proposed technique eluted LIS in 3.97 minutes and hydrochlorothiazide in 4.53 minutes. Lisinopril had a recovery rate of 99.31 to 99.83%, whereas hydrochlorothiazide had a recovery rate of 100.75 to 101.16%. At 1.22 and 0.31 µg/mL, respectively, HCTZ as well as LIS had the lowest detectable values. It was found that the LoQs for lisinopril and hydrochlorothiazide were 0.97 and 3.75 µg/mL, respectively.

Conclusion: It was found that the current RP-HPLC technique is reliable, simple to use, accurate, linear, efficient, and rapid. With a shorter analysis period, this method offers better resolution between the two compounds. Therefore, there is sufficient evidence to include the approach in regular lisinopril and hydrochlorothiazide analysis in a variety of pharmaceutical companies and academic institutions.

Keywords: Developed Method, HPLC, LIS, HCTZ

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.2.09

How to cite this article: Pawar V, More H. Developed and Validated RP-HPLC Method for Concurrent Analysis of Some Cardiovascular Drugs. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):294-297.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The blood pressure-reducing drug angiotensin-converting enzyme (ACE) inhibitors include lisinopril. Structure of LIS has depicted in Figure 1. Blood flow is improved by reducing a substance that typically thickens the blood vessel walls. The generation angiotensin II, vasodilator requires a peptidyl dipeptidase known as ACE." These drugs are prescribed in patients characterized to high blood pressure or heart failure. Stimulating the cortex to block ACE, angiotensin-II lowers plasma angiotensin-I, which in turn lowers vasopressor activity and aldosterone production, which may lead to a transient increase of potassium in the blood.[1,2]

Diuretics like hydrochlorothiazide (HCTZ) are often used. Hydrochlorothiazide causes the kidneys to excrete excess water and salt via the urine, thereby achieving its therapeutic effect. The HCTZ solubility in acetonitrile is shown much lower than that in water. Structure of HCTZ has depicted in Figure 2. As a result of this substance's binding to carbonic anhydrase, it is inhibited. Among the various medical conditions that HCTZ is used to treat are congestive heart failure, high blood pressure, diabetes insipidus, symptom edema, and prevention of stones formation in the kidney.[3-5]

Lisinopril (LIS) may be concurrently calculated using a spectrophotometric method, as the extensive literature review suggested. There are also other spectroscopic, spectrofluorometric, LC techniques for lisinopril determination in pharmaceutical dosage forms and bulk.[6-11] According to our research, spectrophotometry, TLC, flow injection chromatography, and HPLC could be utilised for the identification of hydrochlorothiazide in pharmaceutical formulations, either alone or in conjunction with other drugs.[12-17] Several RP-HPLC technologies have isolated lisinopril from pharmaceutical blends. Although, it has been reported that lisinopril and hydrochlorothiazide can be estimated together in both bulk and medicinal dose form, no RP-HPLC method has been made public. In this study, we present a method for simultaneous measurement of LIS and HCTZ in bulk form as well as dose form using reverse-HPLC testing methods. This

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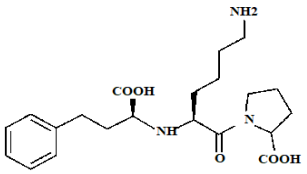

Figure 1: LIS Structure

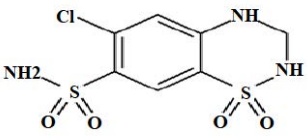

Figure 2: HCTZ Structure

approach is uncomplicated, accurate, quick, and special. The established procedure was shown to be valid according to ICH standards.[18-21]

MATERIALS AND METHODS

Materials

LIS was bought from Micro Labs Limited in Mumbai, India. HCTZ was collected from Cadila Healthcare Ltd. in Sanad, Gujarat, India. We bought commercial tablets of lisinopril and hydrochlorothiazide (100 mg) at a nearby pharmacy. Formic acid and HPLC-grade water were also purchased from Merck Methanol. All of the solvents used here are of the highest calibre and are HPLC-grade. The RP-HPLC system comprises a column made by Phenomenex, USA, Openlab Ezchrom system and other components.

Methods

Chromatographic conditions

A C18 column, methanol: 0.1% formic acid (30:70% v/v), 1 ml/min rate of flow, and 215 nm detection wavelength utilizing a DAD detector were used to create the approach.

Mobile phase preparation

Total of 70% formic acid and 30% methanol make up the prepared mixture. To get rid of any remaining gas the mobile phase was subjected to ultrasonic treatment for ten minutes.

Diluent

We dilute it with the mobile phase.

Preparation of Standard Solution

Standard stock solution of LIS (SSS-L)

Mix 5 mg of LIS with 50 mL of the diluent in a volumetric flask. With extra diluent, increase the total amount to 100 mL. Standard Stock Solution will be this (SSS-L). (LIS Conc. = 50.0 µg/mL).

Standard stock solution of HCTZ (SSS-H)

The following methods are used to produce HCTZ Standard Stock Solution (SSS-H): A 100 mL volumetric flask containing 12.5 mg is filled with fifty mL of diluent, and the liquid is agitated for two minutes before being diluted to a final level of 10 mL. (HCTZ Concentration = 125.0 µg/mL).

Further, addition of five mL diluent, one mL each of SSS-L and SSS-H, a brief vortex, and the final 1-mL of diluent to finish the volume up to ten mL. (LIS Concentration = 5.0 µg/mL and concentration HCTZ was 12.5 µg/mL).

Sample Preparation of HCTZ Ion Pair & LIS Ion Pair

HCTZ Ion pair standard stock solution (SSS-HII): Make a standard stock solution by adding 12.5 mg of HCTZ in volumetric flask, 50 mL of diluent, mixing for 2 minutes, and then diluting with diluent to a final volume of 100 mL (SSS-HII).(Conc. of Hydrochlorothiazide = 125 µg/mL).

To create LIS SSS-LII, add 5.0 mg of the drug to a volumetric flask, mix it with 50 mL of diluent, then add more diluent to bring the volume to 100 mL while stirring for two minutes. (Conc. of LIS = 50 µg/mL).

Add HCTZ 12.5 mg and LIS 5 mg to a volumetric flask to make a solution of standard stock. Add 50 mL diluent and stir for 2 minutes and diluent to bring the volume to 100 mL. Further one mL of this above solution diluted to 10 mL. (LIS concentration of = 5.0 µg/mL & concentration of HCTZ = 12.5 µg/mL).

Product Assay

Solution of tablet formulation (STF)

The average weight of 10 tablets was determined. With a mortar and pestle, the tablets were broken up and combined. Weighed powder of HCTZ 1.25 mg and LIS 0.5 mg was placed in a 10 mL flask; then five mL diluent was taken. This combination further sonicated well for ten minutes; the final volume was made up to 10 mL using diluent, and the final volume was then measured. (Conc. of HCTZ=125 µg/mL, LIS=50 µg/mL). Remove 1-mL of the solution using a pipette, add 5 mL of diluent, vortex, and then add enough diluent to cover the remaining space in a volumetric flask of ten mL flask. (HCTZ =12.5 µg/mL & LIS concentration = 5.0 µg/mL).

RESULTS AND DISCUSSION

Development of Method

It was found that experimenting with different chromatographic settings improved both separation and resolution. With the assistance of a C18 effective separation had accomplished. The LIS and HCTZ peak purity were confirmed by photodiode array detector and observed that the wavelength of 215 nm is sensitive enough to identify both medications. The pH range and solvent ratios tried were extensive, however, the results were unsatisfactory due to either a lack of resolution or a broad peak shape. After many efforts, a superior, crisp peak was achieved with systematic resolution between the peaks when lisinopril and hydrochlorothiazide were separated using HPLC on a C18 column. Retention duration, resolution, sensitivity, and symmetry were all satisfactory.

Method Validation

After the approach was created, it was validated as per official guidelines.

Table 1: Parameters of system criteria

Criteria	*STD Limit*	*For LIS*	*For HCTZ*
RT	-	03.92	4.43
Peak Resolution	Not Less Than 2.0	00.00	2.75
Number of Theo. plates	Not Less Than 2000.0	6632.0	9942.0

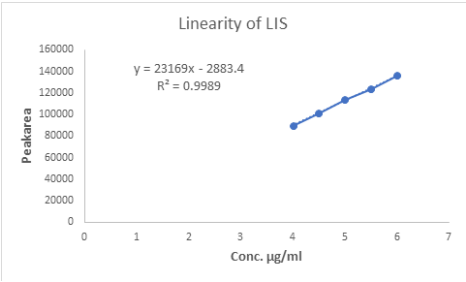

Figure 3: Linearity chart of Lisinopril

System Criteria

A solution of standard was created using the evaluation procedure, it was then fed in HPLC instrument. We looked at how well the system fit our needs in terms of resolution, theoretical plates, and asymmetric components. It was determined that all of the factors were controllable. Table 1 displays the parameters that determine the system's sustainability.

Parameter of Precision

Precision of Method

The precision parameter was studied for the approach proved accuracy. Solution of sample was made and analyzed at the concentration at which the researchers were working. Lisinopril and hydrochlorothiazide sample solution was produced according to the test procedure and injected into the column 5 times. Table 2 displays the accuracy findings. The %RSD was computed and described based on the average. The %RSD readings were determined to be within acceptable ranges, indicating that the method was reliable.

Study of Linearity

LIS and HCTZ standard stock solutions were made in a range of concentrations (from 80 to 120% of the assay concentration) to provide a test solution with known linearity. We utilized a least-squares linear regression to evaluate the relation between peak area and concentration (refer to Figures 3 and 4). The data indicate a substantial peak and their concentration, particularly into 4 to 6 µg/mL for LIS and 10 to 15 µg/mL for HCTZ (Table 3). Given that lisinopril and hydrochlorothiazide had correlation values of 0.9989 and 0.9974, respectively, the linearity of the strategy was established in compliance with the acceptance criteria established by the technique validation.

Accuracy

Three levels was assessed in experiments evaluating the method's efficacy (80, 100, and 120%). Each person took three different tests. Table 4 displays the median and average drug recovery percentages. Increased precision may be to blame for the perceived values being inside the required range, suggesting enhanced recovery value.

Table 2: Data of precision parameter

Number	*For HCTZ*	*For LIS*
Repeatability 1	880220	113954
Repeatability 2	886116	112319
Repeatability 3	869035	113356
Repeatability 4	875485	111229
Repeatability 5	871975	113036
Average	876566	112778
Standard Deviation	6773.986	1049.058
Relative Standard Deviation	0.88	0.73

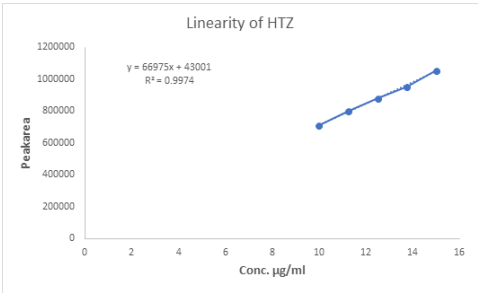

Figure 4: Linearity chart of Hydrochlorothiazide

Table 3: Data of Linearity for LIS and HCTZ

% Level	*Concentration of LIS*	*LIS Peak area*	*Concentration of HCTZ*	*HCTZ peak area*
80	4	89502	10	711306
90	4.5	101310	11.25	801895
100	5	113958	12.5	880225
110	5.5	123899	13.75	953347
120	6	136129	15	1054174

Detection Limit and Quantification Limit

The suggested method has a LoD of a concentration with a S/N ratio of 3.3 and an analytically calculated LoQ of a concentration where the S/N ratio is 10. Therefore, for both medications, a S/N ratio of 3.3 was assigned to the detection limit, and a ratio of 10 was assigned to the quantification limit".

Table 5 displays the data for LoD and LoQ.

HCTZ and LIS tablet Assay

The method was successfully developed as well as validated specifically for the examination of composite pills containing HCTZ and LIS, which may account for its success. The separation achieved by this approach between the two analytes is exceptional, with high resolution. The high percentage of recovery and the absence of influence from the formulation excipients in the pharmaceuticals' retention times further show the technique's selectivity for evaluating the two treatments in their combination dose form. Lisinopril's mean percent approximation was 101.18%, while hydrochlorothiazide's was 99.30%, both of which are far higher than what is stated on the label.

Table 4: Results of LIS and HCTZ for Accuracy

Level Percentage	*% Recovery of LIS*	*Mean Percentage*	*% Recovery of HCTZ*	*Mean (%)*
80	99.41	99.42	100.43	100.56
80	99.42		100.68	
100	100.44	100.02	100.41	100.92
100	99.59		101.09	
120	100.60	99.99	100.10	100.04
120	99.39		99.98	

Table 5: Data of Detection Limit and Quantitation Limit

Sample	Detection Limit in (µg/mL)	Quantitation Limit in (µg/mL)
HCTZ	0.310	0.960
LIS	1.220	3.730

CONCLUSION

It was determined that the current RP-HPLC technique is reliable, straightforward, user-friendly, specific, exact, linear, rapid, and cost-effective. This approach provides better resolution between the compounds in a shorter amount of time than other methods. The technique has therefore been shown to be trustworthy enough to be implemented into the normal assessment of lisinopril and hydrochlorothiazide in a variety of pharmacological circumstances.

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