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RESEARCH PAPER

Development and validation of a reversed-phase HPLC method for the determination of lisinopril and gliclazide in pharmaceuticals

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ABSTRACT

The aim of the present study was to develop and validate a High-Performance Liquid Chromatography (HPLC) method for the determination of lisinopril and gliclazide. The method was developed on Zorbax C_8 analytical column (4.6x250 mm; 5µm) by isocratic elution with a flow rate of 1.0 mL/min and injection volume of 25 µl. The mobile phase composition was methanol:water (65:35 v/v, pH adjusted to 3.0 triethylamine-orthophosphoric acid buffer) and the retention time was found to be 2.883 and 7.456 min for lisinopril and gliclazide, respectively. The developed method was found to be linear in the concentration range of 5-20 µg/mL for lisinopril and 15-60 µg/mL for gliclazide. The method was validated for linearity, accuracy, precision, LOD and LOQ. This developed procedure was succesfully applied conveniently for the analysis of lisinopril and gliclazide in pharmaceutical preparations.

Key Words: Combined dosage forms, gliclazide, lisinopril, RP-HPLC, method validation

Introduction

Diabetes raises the risk of heart disease and other health issues. Having diabetes and hypertension together raises the risk of cardiovascular problems even more. Patients with diabetes should reach a target blood pressure of less than 130/80 mm Hg [1-3].

Diabetes and hypertension are major risk factors for cardiovascular events, and share common pathways such as inflammation, oxidative stress and obesity [4]. For both of these diseases, genetic and lifestyle factors play central roles. It is important to control blood pressure for managing diabetes and many people will require three or more drugs to reach the recommended target.

Gliclazide (GLZ) is classified as a sulfonylurea and acts as an oral hypoglycemic agent used in the treatment of type-II diabetes mellitus [5-7]. It binds to pancreatic sulphonylurea receptors (SURs) on the surface of β-cells, by increasing the secretion of insulin. It also possesses antiplatelet properties with the free radical scavenger activity, by that means prohibiting vascular complications [8, 9].

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Lisinopril (LSP) is an angiotensin-converting enzyme (ACE) inhibitor and a lysine analogue of enalaprilat. Like all ACE inhibitors, lisinopril also displays antihypertensive activity. It is also used for myocardial infarction (MI), heart failure and diabetic nephropathy or retinopathy [10, 11].

Several methods have been previously reported for the determination of lisinopril [12-16] and gliclazide [17-20] by HPLC, UV spectrophotometry, LC-MS, etc. alone but there has been no single method reported for the determination of these drugs together. The aim of our present study was to develop and validate a new rapid and selective HPLC method for the simultaneous determination lisinopril and gliclazide in bulk and combined pharmaceutical dosage forms. The methods have been validated as per ICH requirements [21].

2. EXPERIMENTAL

2.1. Reagents and chemicals

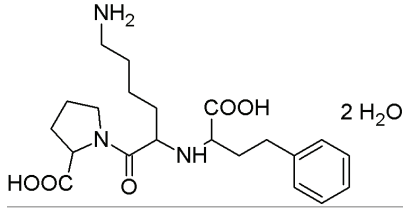
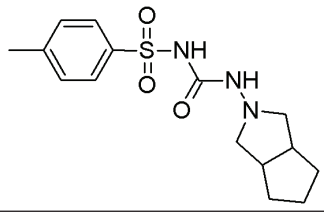
Lisinopril dihydrate was used from the USP reference standard (83915-83-7) and gliclazide was kindly given by Deva Pharmaceuticals. Methanol was of HPLC reagent grade and purchased from Merck (Darmstadt, Germany). Triethylamine (TEA) and orthophosphoric acid (85%) were of analytical grade and procured from Fluka and Carlo-Erba, respectively. HPLC grade water was used to prepare the mobile phase.

2.2. Instrumentation

The HPLC system (Agilent 1100 series), consisted of G 1311A Quat pump, G 1315A DAD detector, a manual injector, with Chem Station A-08,03 [847]-2000 software. Samples were injected with a Rheodyne injector system with a 25 µl sample loop. The detector was set at 215 nm and peak areas were automatically integrated by a computer using the Chem Station software program. Separation was carried out at an ambient temperature using a Zorbax C_8 analytical column (4.6x250 mm, 5µm) by isocratic elution with a flow rate of 1.0 mL/min. The run time was set at 10 min with the system. All the calculations concerning the quantitative analysis were performed with external standardization by the measurement of peak areas.

SMILES codes were generated from the structures using the ACD/ChemSketch version 8.0 molecular editor (http://www.acdlabs.com) and then log P values were calculated using the Molinspiration online property calculation toolkit (http://www.molinspiration.com). The calculated log P values, chemical structures and names, molecular weights and formulas for the drug substances are given in Table 1.

Table 1. Structures and calculated physico-chemical properties of the APIs.

Structures and Chemical Name	Formula & M.W. (g/mol)	Log P*
Lisinopril dihydrate (2S)-1-[(2S)-6-Amino-2-[[(1S)-1-carboxy-3-phenylpropyl] amino]hexanoyl]pyrrolidine-2-carboxylic acid dihydrate 	$C_{21}H_{35}N_3O_7$ 441.518	-2.44
Gliclazide *N*-(Hexahydrocyclopenta [c]pyrrol-2(1*H*)-ylcarbamoyl)-4-methylbenzenesulfonamide 	$C_{15}H_{21}N_3O_3S$ 323.410	1.45

*Log P values were calculated using Molinspiration software (http://www.molinspiration.com/).

2.3. Stock and Standard Working Solutions

A standard solution containing lisinopril and gliclazide was prepared by weighing 10 mg and 30 mg respectively, and both the drugs dissolved in 100 mL mobile phase and the solution was sonicated for 10 min, the volume was made up to the mark with the mobile phase to obtain a stock solution of 100 µg/mL of LSP and 300 µg/mL of GLZ.

LSP and GLZ concentrations in the working solution chosen for the calibration curves were 5, 7.5, 10, 12.5, 15, 20 mg/mL and 15, 22.5, 30, 37.5, 45, 60 mg/mL, respectively. Samples (of low, medium and high concentration) at 8, 10 and 12 µg/mL (for LSP) and 24, 30, 36 µg/mL (for GLZ) were prepared the same way as the calibration. These solutions were freshly prepared daily by making further dilutions of the stock solution in the mobile phase.

2.4. Mobile Phase

Preparation of 0.1% (v/v) triethylamine buffer (pH 3.0): Dissolve 1.0 mL of triethylamine in 900 mL of water then adjust the pH to 3.0 with diluted orthophosphoric acid, and dilute with water to 1 L, filter with Millicup-HV of 0.45-mm pore size.

2.5. Chromatographic Conditions

A HPLC analysis was performed by isocratic elution with a flow rate of 1.0 mL/min. The mobile phase composition was methanol:water (65:35 v/v, pH adjusted to 3.0). The pH of the mobile phase was adjusted to pH 3.0±0.05 with a diluted orthophosphoric acid solution. The mobile was stirred manually to mix and finally make the volume up to 1000 mL with water. Finally the mobile phase was filtered through a 0.45 µm membrane filter and degassed for 10 min. The injection volumes for samples and standards were 25 µl and eluted at a flow rate of 1 mL/min. The retention time was found to be 2.883 and 7.456 min for lisinopril and gliclazide, respectively (Figure 1A). Overlaid UV spectrum showed that both drugs showed good absorbance at 215 nm, hence the wavelength of 215 nm was selected for quantification of LSP and GLZ (Figure 1B). All solvents were filtered through a 0.45 µm millipore filter before use and degassed in an ultrasonic bath.

2.6. Calibration study

Mixed standard solutions containing lisinopril (5-20 µg/mL) and gliclazide (15-60 µg/mL) were prepared in the mobile phase. Triplicate 25 µl injections were made for each standard solution to see the reproducibility of the detector response at each concentration level. The peak area of each drug was plotted against the concentration to obtain the calibration graph. The five concentrations of each compound were subjected to a regression analysis to calculate the calibration equation and correlation coefficients.

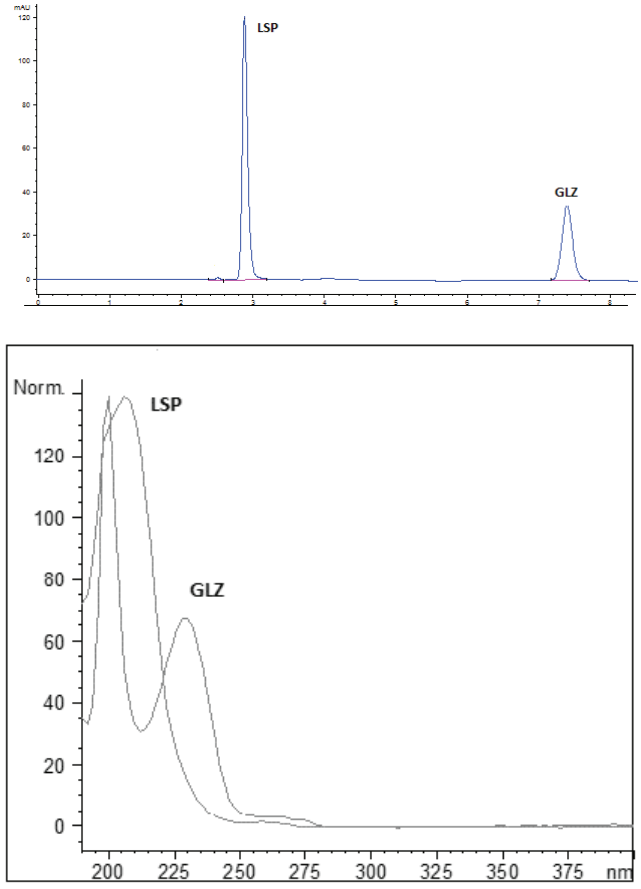


Figure 1. (A) HPLC chromatogram acquired at 215 nm for LSP and GLZ reference standards; **(B)** UV spectra of LSP and GLZ using the mobile phase as blank.

3. RESULT AND DISCUSSION

3.1. Method Validation

The described method was partially validated with respect to system suitability, linearity, limit of detection (LOD) and quantitation (LOQ), precision, accuracy and specificity and selectivity.

3.1.1. Specificity

The specificity of an analytical method is its ability to accurately and specifically measure the analyte in the presence

of components that may be expected to be present in the sample matrix. To investigate the specificity of the analytical method, the blank was injected into the chromatographic system. There was no peak found at the retention time of the analyte in the placebo samples.

3.1.2. System suitability

The system suitability test is an integral part of chromatographic analysis. It is used to verify that the resolution and reproducibility of the system are adequate for the analysis. The results of theoretical plates (N), tailing factor (T), relative standard deviation (RSD) and retention time (Rt) of the method are shown in Table 2. System suitability requirements for drugs were a RSD of peak areas and retention times <1% (by making five replicate injections), a peak resolution (R) >2.0, theoretical plate numbers (N) ≥2000 for each peak and USP tailing factors (T)<1.5. The result was satisfactory.

Table 2. System suitability results of the developed method

Compound	Rt	RSD	R	N	T
LSP	2.883	0.36	2.57	4050	1.38
GLZ	7.456	0.35	19.91	11466	1.09

3.1.3. Linearity and range

Aliquots of standard stock solutions of lisinopril and gliclazide were taken in 10 mL volumetric flasks and diluted with the mobile phase to obtain final concentrations in the range of 5-20 µg/mL for lisinopril and 15-60 µg/mL for gliclazide. The solutions were injected using a 25 µl injection volume into the chromatographic system at a flow rate of 1.0 mL/min and the effluents were monitored at 215 nm, and the calibration curve was obtained by plotting the peak area ratio versus the applied concentrations of the drugs. Triplicate injections were made for each concentration.

Following calibration experiments, concentration levels ranging from 5-20 µg/mL for LSP and 15-60 µg/mL for GLZ were found in linear correlation with the detector response. The correlation coefficients of regression equations were calculated as 0.9994 for LSP and GLZ and shown in Figure 2.

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. The limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ values can be calculated according to the following equations:

$$LOD = 3.3 \times \sigma / S$$
$$LOQ = 10 \times \sigma / S$$

Where "S" is the standard deviation of response of the calibration curve and "S" is the slope of the curve. LODand LOQ values were found to be well within limits. The characterization of calibration plot values is shown in Table 3.

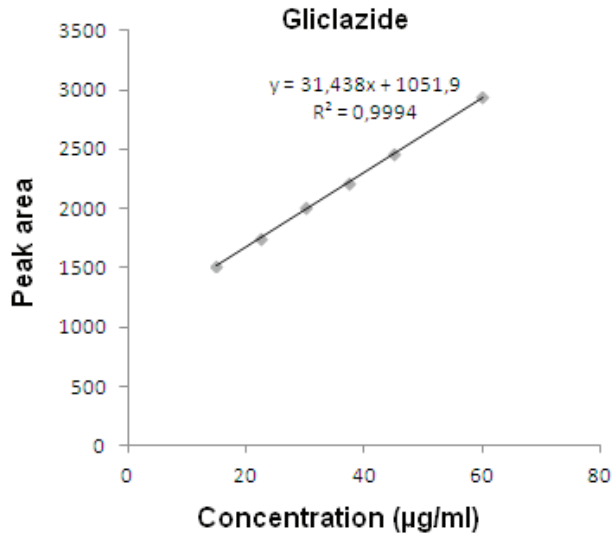


Figure 2. Calibration plots of GLZ and LSP

Table 3. Spectral and statistical data for determination of LSP and GLZ by proposed RP-HPLC method

	LSP	GLZ
Linearity range (µg/mL)	5-20	15-60
Slope*	32.901	31.438
Intercept*	525.67	1051.9
Correlation coefficient (r)	0.9994	0.9994
Limit of detection (µg/mL)	0.46	0.97
Limit of quantification (µg/mL)	1.42	2.95

*: Mean of three injections.

3.1.4. Accuracy

The accuracy was determined with recovery studies. In this validation study, the accuracy of the method has been investigated by calculating the recovery values obtained by analysing the solutions prepared with the lisinopril and gliclazide standard corresponding to 80%, 100% and 120% of the test concentration using the chromatographic parameters defined in the test procedure. The obtained results are presented in Table 4.

Table 4. Results of accuracy study

Test solution	Amount added (µg/ml)	Amount found (µg/ml)	LSP Recovery (%)	Test solution	Amount added (µg/ml)	Amount found (µg/ml)	GLZ Recovery (%)
		8.007	100.09			23.829	99.29
80%	8.0	7.995	99.94	**80%**	24.0	23.988	99.95
		8.112	101.41			24.146	100.61
		9.933	99.33			30.105	100.35
100%	10.0	10.062	100.62	**100%**	30.0	29.973	99.91
		9.978	99.78			30.342	101.14
		11.938	99.49			36.223	100.62
120%	12.0	11.957	99.64	**120%**	36.0	36.101	100.28
		11.996	99.97			36.223	100.62
	Mean		100.03		**Mean**		100.31
	SD		0.64		**SD**		0.54
	RSD (%)		0.64		**RSD (%)**		0.54

3.1.5. Precision

The precision of a method is usually expressed as the standard deviation or relative standard deviation (RSD) of series of measurement.

The system precision or repeatability of the method was checked by repeatedly injecting five sample solutions of LSP (10 µg/mL) and GLZ (30 µg/mL) under the same chromatographic condition and measurements of peak area, retention time and tailing factor. The percentage relative standard deviation (RSD) was found to be <2.0% (Table 5).

Table 5. System precision test results

Test solution	LSP Area	GLZ Area
1	900.6	2056.8
2	906.5	2046.5
3	915.1	2032.9
4	900.9	2068.6
5	907.3	2035.1
Mean	906.5	2047.9
SD	5.91	14.99
RSD (%)	0.65	0.73

The intraday and interday precision of the proposed method was determined by analysing the corresponding responses five times on the same and different days over a period of one week for 100% concentrations of sample solutions of the drugs. The results were reported in terms of relative standard deviation (RSD) (Table 6).

Table 6. Precision study parameter

Lisinopril			Gliclazide		
Results of the intraday precision					
Conc. (mg/mL)	**Peak area (±SD) (n=5)**	**RSD%**	**Conc. (mg/mL)**	**Peak area (±SD) (n=5)**	**RSD%**
10	915.8±7.70	0.84	30	2104.74±20.00	0.95
Results of the interday precision					
10	896.3±6.70	0.75	30	2017.84±14.60	0.72

3.2. Application of the method in dosage forms

The combined dosage formulation of lisinopril and gliclazide was not available on the local market, therefore the laboratory sample mixture was prepared using Rilace® and Diamicron® tablets. Ten equivalently powdered tablets were mixed at a ratio of 10 mg lisinopril: 30 mg gliclazide. A quantity of this synthetic powder mixture equivalent to 40 mg was taken up in a 100 mL volumetric flask, and the mobile phase was added up to the mark. The solution was sonicated for 15 min. This solution was further diluted to obtain a concentration of 10 µg/mL LSP and 30 µg/mL GLZ. The assay results were compiled, found satisfactory and showed that there is a no interference of tablet matrix with the drug and the results are summarized in Table 7. The results show that this method can be easily applied for the estimation of LSP and GLZ in bulk drug and in the tablet mixture.

Table 7. Determination of LSP and GLZ in synthetic tablet blends.

Tablet mixture	Drug	Amount added (mg) in tablet mixture (1:3)	Amount found (mg) (n=3) ±SD	% Amount obtained ±SD
Lisinopril+Gliclazide	Lisinopril	10	9.97±0.18	99.82±0.70
	Gliclazide	30	30.22±0.25	100.34±0.39

4. CONCLUSION

The developed method is suitable for the identification and quantification of the binary combination of lisinopril and gliclazide. The mobile phase is simple and economical to prepare. Validation studies revealed that, the proposed method was found to be rapid, accurate, precise, specific, robust and economical. Hence, the proposed method can be used for routine analyses of both drugs in the process control of the bulk drug and (potential) combined dosage forms without any interference from the excipients in laboratories and in the pharmaceutical industry.

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Farmasötik ürünlerde lisinopril ve gliklazid tayini için ters faz-yüksek basınçlı sıvı kromatografisi yönteminin geliştirilmesi ve validasyonu

ÖZET

Mevcut çalışmanın amacı, lisinopril ve gliklazid etken maddeleri için yüksek performanslı sıvı kromatografisi kullanılarak bir analitik yöntemin geliştirilmesi ve validasyonunu kapsamaktadır. İzokratik ayırım ile sağlanan bu yöntem, 25 mikrolitre enjeksiyon hacmi ve 1.0 mL/dk'ya akış hızı ile Zorbax C8 analitik kolon (4.6x250 mm; 5µm) kullanılarak geliştirilmiştir. Hareketli faz bileşimi metanol:su içermekte olup (65:35 v/v, pH=3'e trietilamin-ortofosfat tamponu ile ayarlı) lisinopril ve gliklazid için alıkonma zamanı sırasıyla 2.883 ve 7.456 dk. olarak bulunmuştur. Geliştirilen bu yöntem lisinopril için 5-20 µg/mL konsantrasyon aralığında, gliklazid için ise 15-60 µg/mL konsantrasyon aralığında doğrusaldır. Yöntem doğrusallık, kesinlik, doğruluk ve LOD-LOQ validasyon parametreleri açısından valide edilmiştir. Geliştirilen bu prosedür lisinopril ve gliklazidin farmasötik preparatlarının analizlerinde güvenli bir şekilde uygulanabilir.

Anahtar Kelimeler: Kombine dozaj formu, gliklazid, lisinopril, ters faz-HPLC, yöntem geliştirme

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