RP-HPLC Method for Simultaneous Determination of Captopril and Diuretics: Application in Pharmaceutical Dosage Forms and Human Serum

Najma Sultana¹, M. Saeed Arayne¹ and Safila Naveed²*
¹Dow University of Health Sciences Karachi, Pakistan
²Faculty of Pharmacy, Jinnah University of Women Karachi, Pakistan

Abstract

An isocratic reversed phase high-performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous determination of captopril and diuretics (furosemide and hydrochlorothiazide) in API, dosage formulations and human serum. Chromatographic separation was achieved on Purospher Start C18 (250 mm x 4.6 mm, 5 μm) and Hypersil ODS C18 (150 x 4.6mm, 5micron) columns using mobile phase, methanol:water (70:30 v/v) adjusted to pH 3.0 via phosphoric acid 85% having flow rate of 1.0 mL min⁻¹ at ambient temperature with detector set at 225 nm. Calibration curves were linear over range of 5-25 μg mL⁻¹ with a correlation coefficient ± 0.999. LOD and LOQ were in the ranges of 0.4-2.3 μg mL⁻¹. Intra and inter-run precision and accuracy results were 98.0 to 102%.

Keywords: Captopril; Diuretics; RP-HPLC

Introduction

Captopril (CAP) belongs to angiotensin II receptor antagonist effective in lowering blood pressure in hypertensive patients. Chemically it is known as (2S)-3-mercaptop-2-methyl-1-oxo-proplyl-L-proline [1]. It has a relatively poor pharmacokinetic profile, while the adverse drug reaction profile of captopril is similar to other ACE inhibitors [2]. In the presence of stenosis, captopril abrogates the protective actions of the high levels of angiotensin II on the autoregulation of the glomerular filtration rate [3]. Captopril does not have a diuretic sparing effect in patients with severe or moderate chronic heart failure. Those treated need the original dose of diuretics for maximal symptomatic benefit. Captopril may be administered with diuretics to manage renal clearance. There have been number of studies using captopril with diuretics [4-13]. Hydrochlorothiazide (HCT) and furosemide (FRS) have widely been used in the treatment of congestive heart failure and hypertension [14,15] (Figure 1).

There are very few methods reported in literature for the simultaneous estimation of angiotensin II receptor antagonist and hydrochlorothiazide in tablets [16-18]. Since these methods were based on HPLC, Capillary electrophoresis and UV derivative spectrophotometry [19,20], the procedures were inconvenient for determination and run time were rather long. Thus, an attempt was made to develop a simple, precise, accurate and economical RP-HPLC method for the simultaneous estimation of captopril furosemide and hydrochlorothiazide in API, pharmaceutical dosage formulations and human serum. Hydrochlorothiazide and furosemide are thiazide and loop diuretics, respectively.

Our research group earlier reported simultaneous quantitation of hydrochlorothiazide with number of other angiotensin II receptor blockers [21]. In present paper, we report a simple, easy, quick and inexpensive isocratic RP-HPLC method with ultraviolet detection at 225 nm for the simultaneous determination of CAP and two diuretics i.e. HCT and FRS. Simultaneous determination of both drugs is desirable as this would allow more efficient generation of clinical data and could be performed at more modest cost than separate assays. The method is equally valid for the determination in bulk materials, pharmaceutical dosage formulations and human serum. This method can be used for the quantitative analysis of diuretics and captopril alone or in combination. The low LOD and LOQ values merit the method for the determination of these drugs in clinical samples.

Materials and Reagents

All chemicals and reagents were of analytical grade. Captopril was a kind gift from BMS (Pvt) Limited, Pakistan. Hydrochlorothiazide and furosemide were gifts from Zafa Pharmaceutical Laboratories (Pvt) Ltd and Sanoﬁ Aventis (Pvt) Ltd Pakistan. HPLC grade methanol and phosphoric acid were obtained from Tedia (USA) and Merck Darmstadt, Germany.

Pharmaceutical dosage form

Capoten™ (Captopril 25 mg tablets by BMS Pharmaceuticals (Pvt) Ltd), Diazu™ (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 (Figure 1). All these drugs had an expiry of not less than 1 year at the time of study.

Instrumentation

The HPLC system consisted of an LC-10 AT VP Shimadzu pump, SPD-10AV VP Shimadzu UV visible detector, a Purospher Start C18 (250 x 4.6 mm, 5μm) and Hypersil ODS C18 columns were used for separation. The chromatographic system was integrated using a CBM-102 communication BusModule Shimadzu with a Pentium™ IV PC loaded with Class GC software for data acquisition. Separation was carried out under isocratic elution with methanol:water (70:30) as mobile phase. The pH of the mobile phase was adjusted to 3.0 with phosphoric acid (85%), sonicated by DGU-14 AM on-line degasser, and filtered through 0.45-micron membrane filter. The flow rate was 1.0 mL min⁻¹, the elution was monitored at 225 nm, and the injection volume was 20 μL.

*Corresponding author: Safila Naveed, Faculty of Pharmacy, Jinnah University of Women Karachi, Pakistan, E-mail: safila117@yahoo.com

Received May 30, 2011; Accepted July 13, 2011; Published July 14, 2011


Copyright: © 2011 Sultana N, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Preparation of standard and sample solutions

**Standard preparation:** Stock standard solutions 100 ppm of CAP, HCT and FRS were prepared in 100 mL mobile phase as solvent. Working solutions were prepared separately by making serial dilutions from the standard solution to obtain concentration between 5-25 μg mL⁻¹ for CAP, HCT and FRS, respectively. These solutions were stored at 20°C. Once prepared, analyzed daily for inter and intra-day variations of the method. 20μL 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 CAP, HCT and FRS was weighed and then dissolved in the mobile phase. Solutions were then filtered through ordinary filter paper. The desired concentrations 2.5, 5, 10, 15, 20 and 25μg mL⁻¹ for CAP, HCT and FRS, respectively were obtained by accurate dilution, solutions were then sonicated. Finally, all the solutions were filtered through 45-μm Millipore™ filter, in order to separate out the insoluble excipients 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 1 min and then centrifuged for 10 min at 10,000 rpm and the supernatant was filtered by 0.45-micron membrane filter. An aliquot of serum sample was fortified with CAP, HCT and FRS to achieve final concentration.

Results and Discussion

Development and optimization of isocratic HPLC conditions

The aim of the present study was to develop a simple, isocratic, accurate and sensitive HPLC method for the simultaneous determination of CAP, HCT and FRS. A UV scan showed a maximal absorbance at near 225 nm. Initial method development was conducted on a Purospher Start C18 (250 x 4.6 mm, 5 μm) column for separation at ambient temperature. This column provides efficient and reproducible separations of non-polar compounds while minimizing solvent usage. Initially various mobile phases were tested to obtain the best separation and resolution. The optimized conditions were reached at pH 3.0, producing well resolved and sharp peaks for all drugs. The specificity of the method was established through the study of resolution factor of captopril peak. For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use and USP 2002 were followed for the accuracy tests, precision, specificity, linearity, work strip and robustness of the method. Retention time of HCT 3.0 min, CAP was 3.3 min and FRS was 4.0 min, at a flow rate of 1.0 mL min⁻¹.

**System suitability**

The HPLC system was equilibrated with the initial mobile phase composition, followed by 6 injections of the same standard. These 6 consecutive injections were used to evaluate the system suitability on each day of method validation. Parameter of system suitability, peaks symmetry (symmetry factor), theoretical plates of the column, mass distribution ratio (capacity factor), and resolution are summarized in Table 1.

**Specificity and selectivity**

The specificity of the chromatographic method was determined to ensure separation of captopril and diuretics as shown in Figure 2. Specificity was also determined by screening four different samples of controlled human serum, which were free from interfering endogenous plasma components. Solutions of placebo, captopril and diuretics were...
prepared and then injected to check for interference from common excipients.

**Linearity**

Linearity is generally reported as the variance of the slope of the regression line. Linearity was tested with known concentrations of CAP, HCT and FRS i.e. 5, 10, 15, 20 and 25 μg mL⁻¹, respectively. Six runs were performed for every concentration [22,23]. Injected concentrations versus area were plotted and the correlation coefficients were calculated which are shown in Table 2.

**Accuracy**

The accuracy of an analytical procedure measures the closeness of measured values to the true values. It was evaluated as percentage relative error between the measured mean concentrations and taken concentrations. Minimal of 3 concentration levels covering the specified ranges were selected and five runs were performed for every concentration and then peak area was calculated as given in Table 3.

**Precision**

For intra-day and inter-day precision, ten samples of five concentrations were analyzed on the same day and after one day (Table 4). Generally acceptable repeatability of the results with in one day and day-to-day was observed. The precision of the method was analyzed as % RSD throughout the linear range of concentrations.

![](image)

**Ruggedness**

The ruggedness was established by determining CAP, HCT and FRS in dosage formulation and in human serum using same and different chromatographic system and two different columns 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 mobile phase composition, flow rate, pH and detection wavelength and it was found that the % R.S.D values did not exceed more than 2% (Table 5).

**Limit of detection and quantitation**

The limit of detection (LOD) and limit of quantitation (LOQ) of this method were determined from the known concentrations of CAP, HCT and FRS. The LOD and LOQ for this assay were calculated which are given in Table 2.

**Conclusion**

The proposed RP-HPLC method for simultaneous assay of captopril, furosemide and hydrochlorothiazide in combined tablets, dosage forms is simple, precise, specific and highly accurate and less time consumption for analysis. So, it can definitely be employed for the routine analysis. Hence this RP-HPLC method is suitable for quality control of raw materials and formulations, and also for human serum. 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, interactions studies and multi-drug pharmacokinetics.

References