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Validation of analytical method for captopril extemporaneous preparations by high performance liquid chromatography

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Abstract

Captopril is commonly used in pediatric patients suffered from hypertension or heart failure. The most convenient and easiest dosage form for administration to these patients is liquid formulation. However, the captopril liquid formulation is not available in the market owing to the instability issue of captopril in liquid media. To accurately and precisely measure the amount of captopril in liquid formulations, the analytical method must be validated. According to the United States Pharmacopeia (USP), the captopril bulk materials and tablet can be quantified by high performance liquid chromatography (HPLC). Therefore, this study adopted the system stated in USP and illustrated the HPLC performance for the analysis of captopril in extemporaneous vehicle. The result found that the appropriate mobile phase consisted of 47% v/v methanol and 53% v/v water containing 0.1% phosphoric acid. From HPLC chromatograms, the retention times of captopril and captopril disulfide were 5.1-5.4 and 12.2-12.9 min, respectively. The linearity was observed over the concentration range of 0.75-20 µg/mL with R^2 of 0.9995. The results confirmed that the system precisely and accurately measured the amount of captopril in vehicle with %RSD less than 2% and %recovery in the range of 97.4-99.8%. The stress test indicated that the HPLC system could distinguishably separate the captopril peak from other degraded products and excipients. Hence, this modified HPLC system could be further used for the chemical stability study of captopril extemporaneous preparations.

Keyword: Captopril; Extemporaneous preparation; HPLC analysis; Method validation

1. INTRODUCTION

Captopril is one of angiotensin converting enzyme inhibitors widely used for the treatment of hypertension and heart failure[1, 2]. It is extensively utilized in pediatric patients suffered from these diseases. Since the commercially available product of captopril is in the form of tablet, these patients require the extremely lower dose of drug than its available strength and are unable to swallow the drug product. Therefore, the extemporaneous preparation of captopril in liquid formulation is essentially needed for these patients in the hospital. Although the captopril liquid formulation has been used for long time, the stability of these preparations has not been well characterized.

In solution, captopril is easily degraded by oxidation reaction which occurs through a combination of autooxidation and metal ion-catalyzed oxidation resulting in captopril disulfide[3]. In addition, it has been reported that an amide linkage of captopril can be hydrolyzed under forced condition. Several factors can affect the degradation of captopril in the liquid form such as pH of solution, oxygen, metal ion, etc. The degraded products of captopril undergone oxidation and hydrolysis are inactive for the treatment in patients. Hence, the amount of captopril in the formulation must be accurately and precisely measured to assure the efficacy of drug for the treatment.

According to the United States Pharmacopeia 35 and the National Formulary 30

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(USP 35-NF 30)[4], the captopril tablet can be assayed by high performance liquid chromatography (HPLC). Many studies have reported the analytical methods for captopril either in combination with other drugs or in the plasma[5-8]. Several reports have coped with the stability of captopril in extemporaneous preparations. Because the extemporaneous preparations are produced and used only in the hospital, the finished products are always different among countries or even hospitals. The differences in sources of drug products and excipients of extemporaneous vehicles may interrupt the accuracy and precision of drug analysis. Therefore, the validation of HPLC analysis is always required prior to its usage for the quantitation of drug in the finished product. Additionally, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline and USP state that the method validation is necessarily performed when a change in either the synthesis of the drug substance, the composition of the finished product or the analytical procedure is made. According to USP, only captopril bulk material and tablet monographs are established but no monograph for captopril liquid dosage form is available. Therefore, the stated HPLC method for captopril tablet was adopted and modified for analysis of captopril in liquid formulations in this study. The analysis of captopril liquid preparation thus meets the criteria for the validation of analytical procedure. This study also reported the performance of extraction method and HPLC system as a quantitative analytical procedure for captopril extemporaneous formulations according to the ICH guideline and USP.

2. MATERIALS AND METHODS

2.1 Materials

Captopril tablet was purchased from Boryung Pharmaceutical Co., Ltd., Seoul, South Korea. Captopril standard was given by the Government Pharmaceutical Organization (Bangkok, Thailand). Sucrose (Mitr Phol Group, Bangkok, Thailand), sodium benzoate (Carlo Erba Reagenti, Milan, Italy) and ortho-phosphoric acid (A.C.S. Reagent, J.T. Baker, Pennsylvania, USA) were used as received. Methanol (Honeywell Burdick & Jackson, Ulsan, Korea) was of HPLC grade. Absolute ethanol was bought from Honeywell Burdick & Jackson, Ulsan, Korea and sterile water for irrigation was obtained from General Hospital Products Public Co.,Ltd., Pathum Thani, Thailand.

2.2 Preparation of standard solution

The captopril standard was accurately weighed into 5-mL volumetric flask and dissolved in a portion of HPLC mobile phase. It was diluted to the volume with the mobile phase. The working standard solution was prepared by appropriate diluting the stock solution with the same solvent. The final concentration of solution ranged from 0.75 to 20 μg/mL.

2.3 Preparation of vehicle

Syrup 80% was used as a vehicle for captopril extemporaneous preparation. The vehicle was prepared as follows. Eighty grams of sugar were weighed and added to deionized water. Then it was heated and stirred continuously for aiding the dissolution of sugar. After completely dissolved, it was allowed to cool down. Then, 0.1 g of sodium benzoate was added and totally dissolved in the syrup. The final volume (100 mL) of vehicle was adjusted by deionized water. The deionized water used in the study was preheated at 100 °C for 30 min prior to the usage.

2.4 Extraction of captopril

To the volumetric flask containing 0.5 mL of vehicle, the accurately weighed amount of captopril standard was added. Then, 90% v/v ethanol was added into the volumetric flask and mixed by vortex mixer. The final volume was adjusted by the same solvent. Subsequently, it was sonicated for 15 min. The precipitates were removed by centrifugation at 4,500 rpm for 15 min. The supernatant (1 mL) was taken and diluted with HPLC mobile phase to 5 mL. The solution was filtered through 0.45 μm nylon syringe filter prior to HPLC analysis. The captopril tablet mixed with vehicle was also

tested for comparison. In this case, the captopril tablet was crushed thoroughly using mortar and pestle and the powder equivalent to 0.5 mg of captopril was accurately weighed into the volumetric flask containing 0.5 mL of vehicle. The extraction of captopril was also conducted in the similar manner.

2.5 HPLC analysis

The HPLC analysis of captopril in extemporaneous vehicle was modified from the method established in USP 35[4]. Captopril was analyzed by Shimadzu HPLC system (DGU-20A5 degasser, LC-20AD pumping system, SIL-20AHT autosampler, SPD-20A UV/VIS detector, Shimadzu Scientific Instruments, Kyoto, Japan). The drug was eluted through Phenomenex® Gemini-NX C18 (110 Å, 5 µm 250×4.60 mm, Phenomenex Inc., Macclesfield, UK) with a guard column (Inertsil® ODS-3, 5 µm, 4.0×10 mm, GL Sciences Inc., Tokyo, Japan). The mixture of methanol and 0.1% v/v phosphoric acid in water (47:53, v/v) was used as an eluent at a flow rate of 1 mL/min. The drug was detected at a wavelength of 220 nm and the injection volume was 20 µL.

2.6 HPLC validation

The quantitation method was validated according to the ICH Harmonization Tripartite Guideline: Validation of analytical procedures: Text and methodology (Q2(R1)) and USP[4, 9]. The requirement for the drug assay follows these topics: system suitability, linearity, accuracy, precision and specificity.

2.7 System suitability

The system suitability was assessed by 5 replicated injections of working standard solution at 100% of test concentration (7.5 µg/mL). The system suitability parameters were computed according to USP[4]. The acceptance criteria stated in the FDA guideline[10] was used to determine the system precision.

2.8 Linearity

The linearity of captopril standard was evaluated over the concentration range of 0.75-20 µg/mL. Three series of six concentrations of working standard solution were prepared and analyzed by HPLC. The standard curve was constructed by the plot between peak area and concentration of the analyte. The slope, y-intercept and the determination of coefficient (R^2) of the regression line were determined.

2.9 Accuracy

The accuracy test was conducted by 3 replications of 3 different spiked concentrations. The %recovery was calculated according to equation 1.

$$\%Recovery = \frac{Measured\ concentration \times 100}{Theoretical\ concentration} \quad (1)$$

2.10 Precision

The precision of HPLC method for captopril was assessed in terms of repeatability and intermediate precision. The repeatability was evaluated by six determinations of the test concentration. The intermediate precision was determined by six determinations of three different analysts. The percentage of relative standard deviation (%RSD) was calculated according to equation 2.

$$\%RSD = \frac{S.D. \times 100}{\bar{X}} \quad (2)$$

where; S.D. = standard deviation

$\bar{X}$ = average value of response

2.11 Specificity

The specificity was evaluated under the stress condition according to the previously published reports[11, 12] as follows. Heat degradation was conducted by incubating the sample at 100 °C for an hour. For hydrolytic degradation, the sample was incubated in an equal volume of either 5 N hydrochloric acid (HCl) solution or 5 N sodium hydroxide (NaOH) solution. After 1-h incubation at room temperature, the sample was neutralized by 2 N NaOH or 2 N HCl, respectively, prior to HPLC analysis. In case of oxidation degradation, the sample was mixed with hydrogen peroxide (0.3%) solution and stored at room temperature for 1 h. Photolytic decomposition was carried out by an exposure of sample to the daylight for a total of 30 h.

3. RESULTS AND DISCUSSION

According to USP, the assay method of commercial product of captopril is available for tablet dosage form by HPLC. The mobile phase for HPLC analysis of captopril tablet consists of 55 %v/v of methanol and 45 %v/v of water containing 0.1% phosphoric acid. By this condition, our result revealed that captopril was eluted at 4.0 min which was interfered with a negative peak (data not shown). Therefore, the concentration of methanol was adjusted to retard the elution of captopril. The concentration of methanol was decreased step by step to 47 %v/v. The result found that the peak of captopril appeared at around 5.1-5.4 min and it was not overlapped with any other peaks (Figure 1).

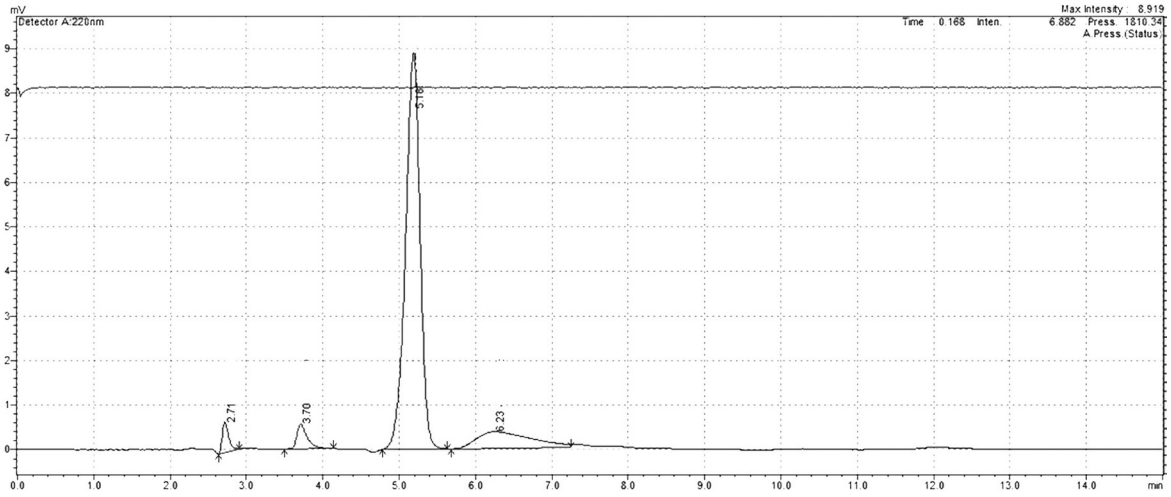

Figure 1. Example of HPLC chromatogram of captopril at a concentration of 7.5 µg/mL

System suitability was determined by calculating the relative standard deviations (%RSD) of area and retention time from five replicate injections of 7.5 µg/mL captopril solution. As summarized in Table 1, the %RSD values of area and retention time were less than 2% indicating the precise analysis of captopril by this system. The resolution of captopril was larger than 2 suggesting the captopril peak could be well separated from other peaks. The tailing factor was closed to 1.0. From our results, one peak appeared at 12.2-12.9 min and it was expected to be captopril disulfide. As compared to the monograph, the ratio of methanol and acidic water used in our system was slightly decreased from 55:45 to 47:53. This modification of composition of mobile phase fell within the limit of maximum specification for method modification[4, 10]. Our attempt was further made to confirm the peak of captopril disulfide. It has been widely known that the main degradation product of captopril is captopril disulfide and occurs through oxidation reaction (Figure 2)[3]. Hence, the oxidation of captopril was performed by incubating the captopril solution with an excess amount of hydrogen peroxide overnight at room temperature. Thereafter, the solution was analyzed by HPLC. The HPLC chromatogram detected a dramatic increase in intensity of the peak at 12.94 min with a small peak of captopril at 5.38 min as shown in Figure 3. Therefore, it could be implied that the peak at 12.2-12.9 min was captopril disulfide. According to the captopril monograph in USP 35, it is stated that the relative retention times of captopril and its degraded product, captopril disulfide, were 0.5 and 1.0, respectively. From our results, the relative retention times of captopril and captopril disulfide were 0.4 and 1.0, respectively.

Since the assay of captopril liquid dosage form is not available in the pharmacopeia, the adapted HPLC method from captopril tablet monograph in USP is necessarily validated.

According to USP and ICH Q2(R1) guideline, the requirement for validation of HPLC analysis includes linearity, accuracy, precision and specificity[4, 9, 13]. The concentration range of captopril standard curve was 0.75-20 µg/mL. Three series of six captopril concentrations were individually analyzed. The responses of all three series were plotted against their actual concentrations and the slope, y-intercept and coefficient of correlation were computed as illustrated in Figure 4. The standard curve was acceptably linear with R^2 of 0.9995.

Table 1. System suitability parameters of captopril solution

Number of Replication	Area (mV)	Retention time (min)	Resolution	Number of Theoretical plate	Tailing factor
1	115881	5.18	4.79	3238	0.93
2	116743	5.21	5.38	3436	0.92
3	117065	5.22	4.52	3444	0.92
4	118269	5.30	5.52	3459	0.92
5	118571	5.38	4.73	3386	0.90
Mean	117306	5.26	4.99	3393	0.92
S.D.	1111	0.08	0.44	91	0.01
%RSD	0.95	1.55			
Criteria10	< 2%	< 2%	> 2	> 2000	< 2

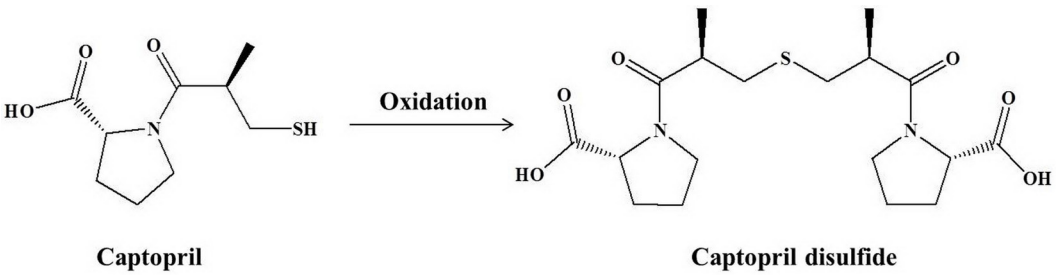

Figure 2. Degradation pathway of captopril by oxidation reaction

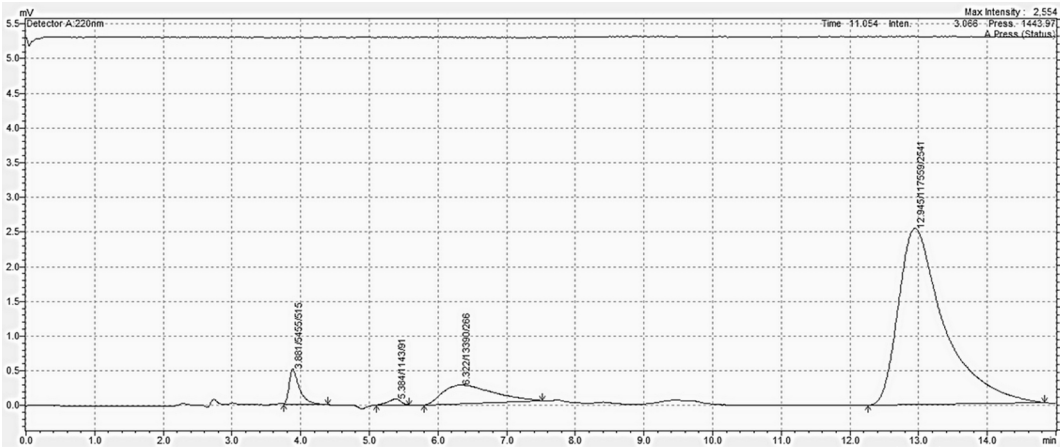

Figure 3. HPLC chromatogram of captopril solution after incubating with an excess amount of hydrogen peroxide

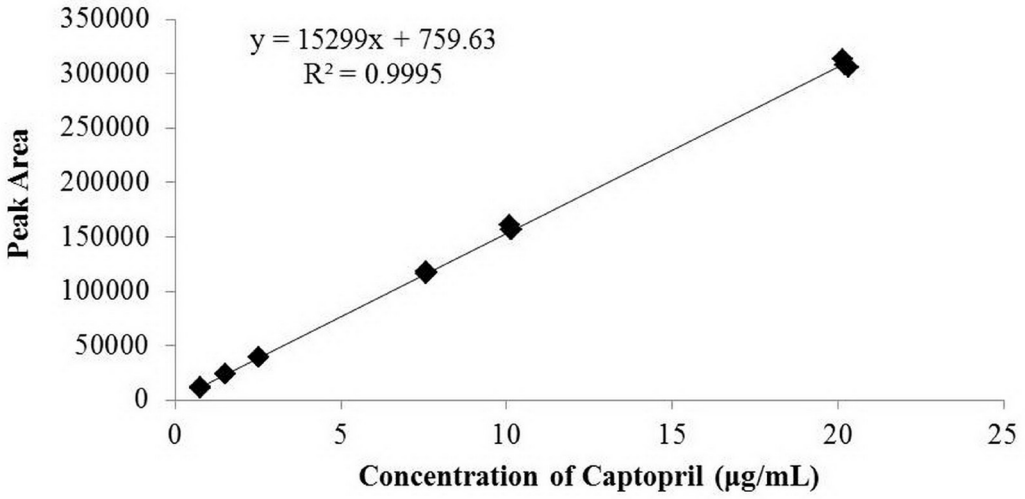

Figure 4. Standard curve of captopril over the concentration range of 0.75-20 µg/mL

In case of precision, the repeatability and intermediate precision were evaluated and the results are summarized in Table 2. The measured concentration of repeatability was 7.53 ± 0.11 µg/mL with %RSD less than 2.0%. The intermediate precision was assessed by three analysts. The results found that %RSD was smaller than 2.0% for all measurements. The accuracy was also determined from three different concentrations. The %recovery was recorded in Table 2. The result discovered that the %recovery was in the range of 97.4-99.8%. The extraction of captopril tablet from vehicle was also tested. The result revealed that captopril could be completely extracted from the vehicle and was comparable to the captopril tablet in mobile phase (data not shown). According to the HPLC chromatograms (Figure 5), no peak of vehicle interrupted the eluted captopril.

Table 2. Repeatability, intermediate precision and accuracy results of HPLC method for captopril analysis

	Precision		Accuracy		
	Repeatability	Intermediate precision			
Spiked concentration (µg/mL)	7.5	7.5	1.0	7.5	15.0
%RSD	1.46	1.60	-	-	-
%Recovery	-	-	98.4	99.8	97.4

For the specificity test, the captopril standard and tablet were mixed with vehicle and subsequently subjected to the stress test under heat, acid, base, oxidation and daylight conditions. As displayed in Figure 5, captopril was separately eluted at 5.3 min without any interference from other peaks. The peak at 9.6 min attributed to sodium benzoate which was confirmed by the HPLC chromatogram of vehicle. Under these forced degradation conditions, captopril was evidently degraded by acid, base and oxidation. The appearance of the peak at 12.9 min in HPLC chromatogram of oxidation condition was attributable to captopril disulfide. Comparing with captopril powder, the similarity of HPLC chromatogram

of captopril tablet in vehicle was observed. Under the stress conditions, the drug from captopril tablet in vehicle was significantly degraded through acid-catalyzed hydrolysis, base-catalyzed hydrolysis and oxidation. The HPLC chromatograms showed a good separation of captopril from other peaks. The result indicated that the modified HPLC condition could distinguish the captopril peak from other degraded products and excipients.

According to the USP monograph, captopril disulfide is recognized as the impurity in captopril material and product and its level in the drug material and product is limited. Therefore, the specificity test and limit of detection of captopril disulfide are required. From HPLC chromatograms, the peak of captopril disulfide was distinguishable from other peaks. Based on signal-to-noise ratio, the limit of detection of captopril disulfide was 0.07 μg/mL.

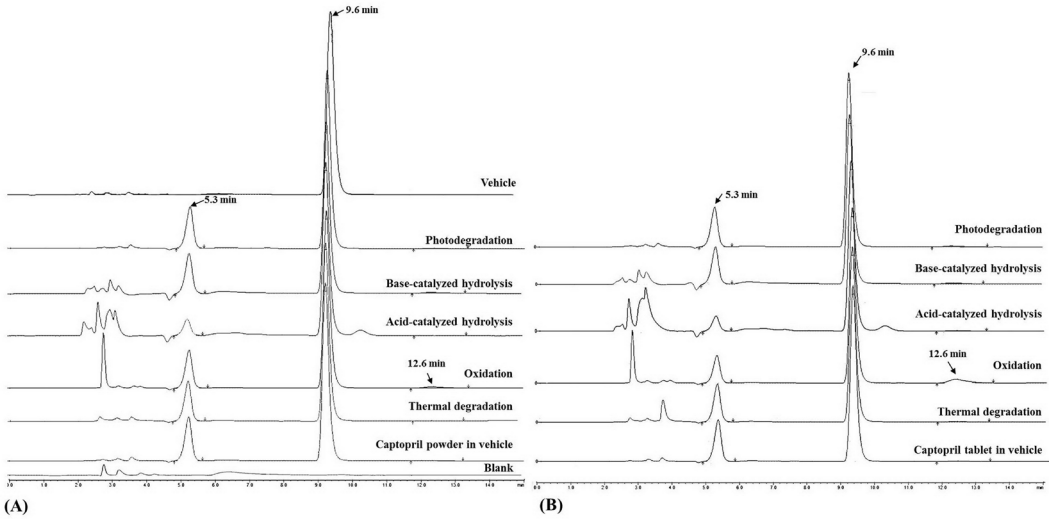

Figure 5. HPLC chromatograms of captopril powder (A) and tablet (B) after subjected to the stress test

CONCLUSION

The modified HPLC system could be applied for the analysis of captopril from the extemporaneous vehicle used in this study. Captopril was precisely and accurately quantified and its degradation product could be identified. This HPLC condition and extraction method will further be used for the stability study of captopril extemporaneous preparations.

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