ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 11, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

DEVELOPMENT OF ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF CAPTOPRIL IN PHARMACEUTICAL DOSAGE FORMS

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Received: 05 June 2017, Revised and Accepted: 02 August 2017

ABSTRACT

Objective: The objective of this research was to develop more simple, sensitive, accurate, and less expensive analytical methods for the determination of captopril in medicines by Ultra-high-performance liquid chromatography.

Materials and Methods: The chromatographic analysis of captopril performed on liquid chromatography Agilent 1290 Infinity II LC System.

Results: A simple, rapid, sensitive, and specific method was developed for the determination of captopril by ultra-high-performance liquid chromatography in mono-medicines and pharmaceutical dosage forms in combination with hydrochlorothiazide without previous separation. Satisfactory resolution was achieved using Fused-Core® technology Ascentis Express C18 column (4.6×150 mm) and a mobile phase consisting of methanol and 0.1% solution of trifluoroacetic acid (40/60, v/v) at a flow rate 1.2 mL/minute and the wavelength detection was 220 nm. Ascentis Express columns, based on Fused-core particle technology, provide more than twice the speed and efficiency of traditional columns at half the backpressure of sub-2-µm columns. The retention time for captopril was 1.345 minute. The validation of this method was based on the ICH and USP guidelines.

Conclusion: The results obtained in this research work clearly indicated that the assay was rapid, sensitive and successfully applied to the determination of both drugs in pharmaceutical dosage forms without interference from tablet excipients.

Keywords: Captopril, Hydrochlorothiazide, Ultra-high-performance liquid chromatography, Pharmaceutical dosage forms.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i11.20462

INTRODUCTION

Captopril, 1-[(2S)-3-mercapto-2-merhyl-1-oxopropyl]-L-proline, (2S)-1-[(2S)-2-Methyl-3-sulphanylpropanoyl]pyrrolidine-2-carboxylic acid, is a sulfhydryl-containing analog of proline with antihypertensive activity and potential antineoplastic activity. Captopril competitively inhibits angiotensin-converting enzyme, thereby decreasing levels of angiotensin II, increasing plasma renin activity, and decreasing aldosterone secretion. Hydrochlorothiazide, 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide is used as a diuretic [1-3]. Captopril is official in EP and USP; hydrochlorothiazide is official in BP and EP. Captopril presents in pharmaceutical market in tablets 25 mg. Combination of captopril and hydrochlorothiazide is marked as combined dose tablet formulation in the ration of 50:12.5 mg and 50:25 mg (Captopres).

A literature survey was conducted, and several methods were reported for the determination captopril, such as spectrophotometry, liquid chromatography, and capillary electrophoresis [4]. However, no rapid and sensitive ultra-high-performance liquid chromatography (UHPLC) for the determination of captopril in pharmaceutical dosage forms.

Our aim was to develop a simple, rapid, sensitive, and specific method for the determination of captopril in mono-medicines and pharmaceutical dosage forms in combination with hydrochlorothiazide without previous separation.

MATERIALS AND METHODS

Materials and reagents

USP grade CPT and USP grade HCT were kindly donated by Darnitsa, Kiev, Ukraine. Solvents purchased from Merck, Darmstadt, Germany HPLC grade. Tablets Captopril was kindly provided by Ternofarm, Ternopil, Ukraine. Tablets Captopres was kindly provided by Darnitsa, Kiev, Ukraine.

Apparatus

Agilent 1290 Infinity II LC System. Fused-Core® technology Ascentis Express C18 column (4.6×150 mm).

Chromatographic conditions

Chromatographic analysis was performed at ambient temperature (22°C-25°C). The compounds were separated isocratically with a mobile phase consisting of methanol and 0.1% solution of trifluoroacetic acid (40/60, v/v) at a flow rate 1.2 mL/minute with injection volume 2 µL. The effluent was monitored spectrophotometrically at wavelength 220 nm. Column temperature was 35°C.

Preparation of stock solutions

Primary stock solutions of captopril were prepared daily separately by dissolving 25 mg of each in 25 mL volumetric flasks (1.0 mg/mL) in the mobile phase.

Procedures

Triplicate 2 µL injections were made for each concentration and chromatographed under the condition described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph, and regression equation was computed.

Preparation of pharmaceutical dosage sample for tablets captopril

The contents of 20 tablets labeled to contain 25 mg of captopril were individually weighed, mixed and powdered. Amount of the powder

equivalent to two tablets content was accurately weighed, transferred into 50 ml volumetric flask and diluted with mobile phase. The sample solution was then filtered using 0.45 µm filters (Millipore, Milford, MA).

Preparation of pharmaceutical dosage sample for tablets captopres

The contents of 20 tablets labeled to contain 50 mg of captopril and 12.5 mg of hydrochlorothiazide were individually weighed, mixed and powdered. Amount of the powder equivalent to one tablet content was accurately weighed, transferred into 50 ml volumetric flask and diluted with mobile phase. The sample solution was then filtered using 0.45 µm filters (Millipore, Milford, MA).

Calibration and linearity

Triplicate 2 µL injections were made for each working standard solution. The peak area for each concentration was recorded and then plotted against the corresponding concentration to obtain the calibration graph.

RESULTS AND DISCUSSION

In this study, our first trials were directed to find optimal chromatographic conditions [5-10]. Our objective of the chromatographic method development was to achieve a peak tailing factor <1.5, retention time in between 1 and 3 minutes, along with good resolution. Ascentis Express columns, based on Fused-Core particle technology, provide more than twice the speed and efficiency of traditional columns at half the backpressure of sub-2-µm columns. This objective was obtained using mobile phase consisting of methanol and 0.1% solution of trifluoroacetic acid (40/60, v/v). The mobile phase composition was optimized under the described conditions; the analyte peaks were well defined, resolved and free from tailing, the tailing factors were <1.5 for all peaks. The elution orders were hydrochlorothiazide (tR 1.05 minute), and captopril (tR 1.345) at a flow rate of 1.2 mL/minute. The optimum wavelength for detection was 220 nm at which much better detector responses for the drugs were obtained (Fig. 1-3).

Method validation

Linearity, detection, and quantitation limits

Calibration curve representing the relation between the concentrations of drugs and the peak area was constructed. In triplicate run from which the linear regression equation was calculated [11,12]. The statistical quantities *b, a, Sr* (final standard deviation) calculated and *r* (correlation coefficient) were listed in Table 1. The limit of detection and limit of quantitation were experimentally determined were also presented in Table 1. The results obtained were statistically processed by the least squares method according to the requirements of ICH recommendation. The results obtained were processed by the least-squares method for line $Y=m\times x+b$. The values of correlation coefficient were close to unity indicating good linearity. Results indicate a high sensitivity of the proposed UHPLC method.

Accuracy and precision

Accuracy and precision were studied by «added-found» on standard solutions of captopril (Table 2). Model solutions were prepared according to the procedure completely repeating the procedure for preparing the test solution. By comparing the two solutions for each analyte built calibration graph (level 1-2, including all parallel injection and specifying the appropriate concentration reference solution), passing through zero.

The relative standard deviation of drugs peak area in five triplicate injections of standard drug solution determined each day of 3 consecutive days. Intraday and interday precision were assessed using three concentration and three replicates of each concentration. The calculated relative standard deviation values were found to be small below 2% indicating good repeatability and reliability of the proposed UHPLC method. The results and their statistical analysis were summarized in Table 3.

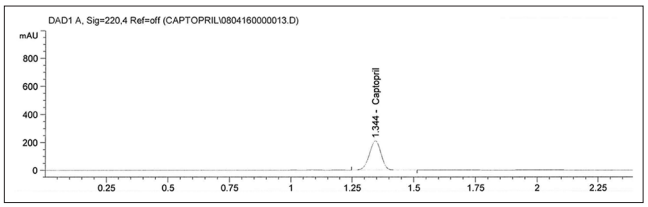

Fig. 1: Representative chromatogram of USP captopril

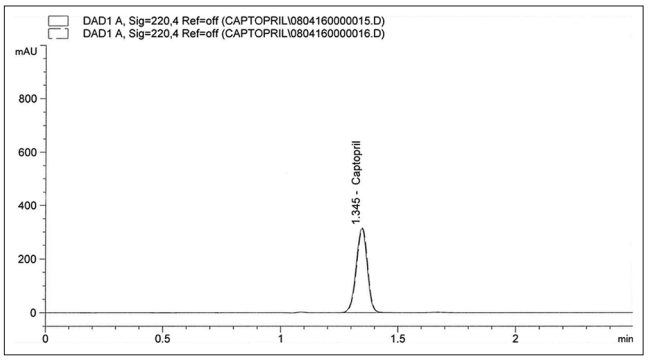

Fig. 2: Representative chromatogram of captopril in tablets captopril

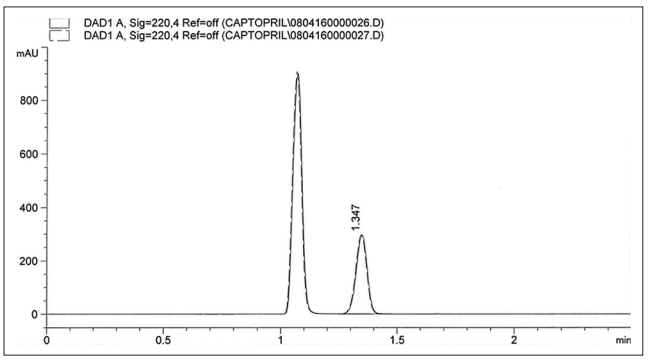

Fig. 3: Representative chromatogram of captopril in tablets captopres

Table 1: Characteristics of the linear dependence of captopril

The slope of the linear relationship *b*	−1.29023e-1
The constant term of the linear dependence *m*	991.36185
The residual standard deviation *Sr*	0.32620
The correlation coefficient method *r*	0.99999
LOD (µg·mL)	5
LOQ (µg·mL)	10

LOD: Limit of detection, LOQ: Limit of quantitation

Table 2: Evaluation of the accuracy of captopril by the proposed UHPLC method

Sample No.	Taken µg/mL	Found µg/mL	% Recovery
1	10.0	9.89	98.9
2	16.0	16.16	101.0
3	20.0	20.22	100.2
4	25.0	25.15	100.48
5	30.0	29.78	99.26
Mean			99.96
±SD			0.86
±RSD			0.86

SD: Standard deviation, RSD: Relative standard deviation, UHPLC: Ultra-high-performance liquid chromatography

Table 3: Evaluation of the precision of captopril by the proposed UHPLC method

Theoretical concentration (µg/mL)	Mean±RSD%	
	Intraday measured concentration (µg/mL)	Interday measured concentration (µg/mL)
16	99.82±0.311	100.76±0.364
25	100.41±0.647	99.27±0.390
30	100.82±0.336	100.53±0.572

RSD: Relative standard deviation, UHPLC: Ultra-high-performance liquid chromatography

Table 4: Evaluation of the robustness of captopril by the proposed UHPLC method

Conditions of analysis	Retention time, minute
Standard conditions	1.345
Flow rate 1,3 ml/minute, (+10%)	1.712
Flow rate 1,1 ml/minute, (−10%)	1.687
Temperature of column 38°C	1.497
Temperature of column 32°C	1.489

UHPLC: Ultra-high-performance liquid chromatography

Specificity

Retention time of the peak in the chromatogram of tablets was the same as that of standard drugs without interference from excipients. Results complited in Tables are satisfactory in terms of accuracy and precision which confirms that excipients did not interfere with the determination.

Robustness and ruggedness

Robasnist - resistance techniques to small changes in experimental conditions tested in the test solution. Terms chromatography varied within±10% of these in the procedure. The research results were listed in Table 4.

Most of the results affected by changes in the flow rate of the mobile phase, but they are insignificant. Temperature changes in terms of column chromatography virtually no effect on the analysis.

CONCLUSION

The proposed UHPLC method was specific and easy to perform allowing rapid determination of captopril in mono-medicines and pharmaceutical dosage forms in combination with hydrochlorothiazide. Validation of the proposed procedures was carried out according to the ICH and USP guidelines.

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